STUDIES ON PLANT PARASITIC NEMATODES IN RELATION TO SOIL POLLUTANTS

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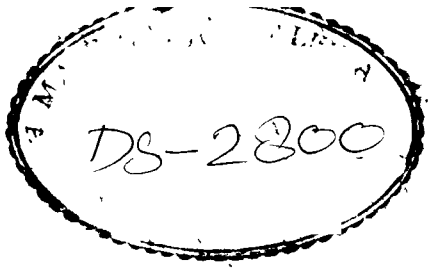
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CERTIFICATE

This is to certify that the dissertation entitled "Studies on plant parasitic nematodes in relation to soil pollutants" is a faithful record of the bonafide research work carried out by Miss Shefta Jasneem Chandel under my supervision and guidance. Her work is up-to-date and original. She is allowed to submit the dissertation to the Aligarh Muslim University, Aligarh, for consideration of the award of the degree of Master of Philosophy in Agriculture (Plant Nematology).

M. M. Alam

(M. Mashkoor Alam)

DEDICATED
TO MY
PARENTS

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INTRODUCTION

1. INTRODUCTION

Plant-parasitic nematodes are minute, vermiform, multicellular and non-segmented invertebrate animals with simple body organization. They are cosmopolitan in distribution and are one of the important limiting factors of plant growth and productivity. They cause great destruction to plants either singly or in collaboration with other pathogens and parasites. Nematodes incurr losses not only in the form of reduced plant growth and yield but also in the marketable quality of the products. It is difficult to assess economic losses they cause to crop plants. It was observed that Heterodera cajani caused heavy damage to Sesamum fields in Jaipur district, Rajasthan, (Sharma and Trivedi, 1993). The root-knot nematodes have been reported to cause 28.08, 33.68, 43.48, 28.60 and 19.95 percent loss in yields of okra, brinjal, french bean, cowpea and peas, respectively (Parvatha Reddy and Singh, 1981). On an average crop losses caused by plant-parasitic nematodes alone have been assessed to be to the extent of 10 percent annually (Anon, 1971).

Hutchinson et al. (1961) estimated a loss of $ 250 million due to plant-parasitic nematodes. Later, Taylor (1967) reported an annual crop loss of $ 372, 335,000 in vegetables due to nematodes. According to Antonio (1988) a yield reduction of soybean was found to be 55.6% due to Meloidogyne incognita in Brazil. An assessment of losses in England and Wales in 1949, revealed that Globodera rostochiensis alone was responsible for causing losses worth of 200,000 tones (Southey and Samuel, 1954). The annual reduction of 5 million Kroners caused due to cereal cyst nematode Heterodera avenae in Denmark was estimated by Stapel (1953). The Society of Nematologist Committee had estimated 11 percent annual loss in vegetables ($267 million per year) due to nematodes in U.S.A. (Feldmesser et al., 1971). A similar Committee (Anon, 1971) reported that annual losses due to nematodes were 6 percent in field crops ($110 million per year), 12 percent in fruit and nut crops ($225 million per year) and 10 percent in ornamentals ($60 million per year). Van Berkum and Seshadri (1970) estimated annual losses due to plant-

parasitic nematodes in India in few crops. According to them, Heterodera avenae caused $ 8 million loss to wheat and barley crops in Rajasthan province alone, Anguina tritici $ 10 million to wheat crop and Pratylenchus coffeae $ 3 million to coffee. According to Sasser's general estimate of losses in vegetables caused by root-knot nematodes in different regions of the world vary from 11 to 25% (Sasser, 1979). Sasser and Freckman (1987) have indicated an annual crop loss due to plant-parasitic nematodes on world wide basis to the tune of $ 100 billion.

The main sphere of activity for most of the plant- parasitic nematodes is soil. Even the aerial parasites also have a soil phase in their life cycle. Various physical and chemical factors of the soil affect the population of nematodes in the soil.

Rapid industrialization has resulted in the accumulation of sewage sludge, industrial wastes and fertilizer impurities containing various heavy metals and organic pollutants. The translocation of some heavy metals such as Hg, Cd, Cu, Co, Ni and Zn is affected by organic pollutants, i.e. methanol, ethanol, propanol,

acetone, formaldehyde, benzaldehyde, ethyl methyl ketone and cyclohexone. These organic compounds are also added to the soil as a result of decomposition of organic matter in the presence of microorganisms. The important organic compounds released are carboxylic acids, formic acid, oxalic acid, phenols, etc. These organic compounds form complexes with soil organic matter, humic acid, fulvic acid, etc. and affect the mobility and toxicity of heavy metals. The presence of organic and inorganic pollutants in sewage wastes restricts their use in agriculture due to toxic metals accumulation in soil.

Heavy metals find their way into the environment through various natural and man made sources, and lead to several undesirable effects on water, air and soil components of the environment. Volatilization of metals from metallurgical industries can give rise to localised pollution by metals initially in the atmosphere and then on plants, soil and water by wet or dry deposition of metallic compounds.

Toxic metals to a large extent are dispersed in the biosphere. They can be carried to places far away from the source by wind depending on whether they are in gaseous or particulate form. Metallic pollutants are ultimately washed out by rain on to land or the surface of water ways. Metals contained in industrial effluents constitute a major source of metallic pollution of hydrosphere. This discharge into river water leads to concentration of toxic elements within food chains in marine eco-system.

Metals entering the soil constitute a more lasting form of pollution. The potential pathways of the metals present in the soil are uptake by plants, movement with water to ground or surface water sources, and immobilization in the soil matrix. Heavy metals applied to soil may pass through the soil unchanged, react with organic or inorganic compounds to form soluble or insoluble compounds, be adsorbed on soil colloids, volatize from the soil or be taken-up by plants (Epstein and Chaney, 1978). Uptake of heavy metals by plant can continue long after the pollution ceased. Electroplating, metal processing and refining, storage battery manufacturing and tanning industries,

etc. are mainly responsible for heavy metal pollution.

The term 'heavy metals' is often used where there are cannotations of toxicity. "Pollution" is the contamination of air, water and soil with harmful chemicals and radiations. The chemicals and radiations whose presence causes pollution are called "pollutants". Wood (1968) classified air pollutants into two categories based on their origin, viz. primary air pollutants and secondary air pollutants. Primary air pollutants are those that originate directly from the sources. Such air pollutants may be in gaseous or particulate forms. Gaseous air pollutants are oxides of nitrogen, NH_3, SO_2, flouride, CO_2, carbon monooxide etc. The particulate air pollutants are cement dust, soil dust, suspended particulate matter, flyash, coal dust, etc. The secondary air pollutants are produced by the reaction of primary gaseous air pollutants, e.g. O_3 and peroxyacetyl nitrate (PAN). Some gaseous air pollutants like SO_2 and NO_X in high humid conditions are converted into acid (H_2SO_4 and HNO_3) which fall on the ground in the form of 'acid rain' during the atmospheric precipitation (Oden, 1968).

The role of air pollutants on crop plants are being realised now in different parts of the world (Heck et al., 1986). Growth, biomass, yield of the crop plant and trees are adversely affected by air pollutants (Mudd and Kozlowski, 1975; Heggested, 1988; Heck et al., 1986). Gaseous air pollutants enter the leaves through stomata which cause injury directly in the leaf tissue or interfere in the bio-chemical reactions (Pell, 1979). In nature, interaction between biotic plant pathogens and air pollutants (abiotic pathogens) may also develop under specific conditions (Pell, 1979).

Air pollutants produce visible symptoms like necrosis, stunting, early senescence and chlorosis, etc. and also affects the physiology and biochemistry of the plant (Heagle, 1973). Nickle toxicity is a recognized problem in agricultural areas with high soil nickle levels (Frank et al., 1976; Hunter and Vargnano, 1952, Lagerwerff, 1967)

Several reports of effects of pollutants on plant pathogens have been published (Heagle, 1973), but relatively few have concerned nematodes. Areas of forest severely damaged by sulfur dioxide (SO_2) and

alkaline particulates were found to have higher nematode populations than less damaged areas (Bassus, 1968). Ozone and SO_2 inhibited reporduction and development of certain nematodes species on soybean (Weber et al., 1979). Studies with cysts nematodes (Heterodera spp.) indicated that several inorganic ions, including Ni^{++} stimulated hatching of eggs (Clarke and Shepherd, 1966). In a greenhouse bioassay root-knot nematode (Meloidogyne hapla) retarted growth of lettuce leaves by 50% in metal contaminated soil and 30% in control soils (Temple and Bisessar, 1981). This aspect, constituting the focal theme of the present study, has been reviewed in the next chapter.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Industrial effluents wastes, present in the soil and water, contain toxic materials which cause harmful effects on plant life (Schraufnagel, 1962; Ajmal and Khan, 1984; Ajmal et al., 1984). Hutchinson and Whitby (1974) and Lagerwerff and Brower (1974) reported soil pollution due to heavy metals near the industries. Gotoh et al., (1979) reported that mercury is also found in sufficient amount to cause pollution in the soil which is derived from the igneous rocks. Katsuhiro and Shigenori (1969) found that mercury retained in the soil by its colloidal particles. The roots absorb the heavy metals which are translocated to different parts of the plant (Haghiri, 1973; Lagerwerff, 1971). The uptake of these soil pollutants is influenced by soil factor like cation exhcange capacity, pH, availability of phosphorus, etc. (Miller et al., 1976). Heavy metals also affect the plants (Tyler, 1974; Vaituzis et al., 1975). Many workers have reported harmful effects on the growth and yield of crop plants due to industrial effluents, waste materials and heavy metals (Froster,

1954; Hassett et al., 1976; Beckett and Davis, 1978; Hale et al., 1985; Ajmal et al., 1984).

Industrial dairy processing effluent reduced the growth of kidney bean and pearl millet (Ajmal et al., 1984). Ajmal and Khan (1984) irrigated wheat and pea with the effluent from Mohan Meakin Breweries Ltd., Ghaziabad, U.P. and found reduction in the germination and growth when irrigated with 100% effluent. However, irrigation with 50% was beneficial to plant growth. Goodbold and Hutterman (1985) observed that mercury was much toxic in inhibiting root elongation in comparision of zinc and cadmium. Similar results were also observed by Graft and Schwantes (1983).

INFLUENCE OF AIR POLLUTANTS ON NEMATODES

Review on the work concerning the effects of air pollutants, such as SO_2, O_3, NH_3, ash, etc. on plant-parasitic nematodes is presented here under:

Sulphur dioxide and Ozone (SO_2 and O_3):

Bassus (1968) reported that saprophagus and predacious nematodes were severely damaged by SO_2 and

alkaline particulate materials in the forest area. Weber et al. (1979) studied the effect of O_3 and SO_2 on the reporduction of five species of plant-parasitic nematodes with different feeding habits. Exposure of infected soybean plants to O_3 and O_3-SO_2 mixture inhibited reproduction and development of Heterodera glycines and Paratrichodorus minor, while Belonolaimus longicaudatus remained unaffected. Exposure of soybean host plants to SO_2 enhanced the reproduction of Pratylenchus penetrans compared with that in plant exposed to the charcoal filtered air (control) or to O_3. Foliar injury of begonia by O_3 or O_3-SO_2 mixture inhibited the increase of Aphelenchoides fragariae. The suppressive effects of A. fragariae were greater in leaves pre-exposed to O_3 or O_3-SO_2 mixture before rather than after leaves were inoculated with nematodes. Growth of nematodes in infested soybean plant and leaves of begonia was inhibited by O_3 and O_3-SO_2 mixture compared with that of similar control plants grown in the presence of nematodes and charcoal filter air. Ozone and O_3-SO_2 mixture suppressed the nodulation of soybean plants inoculated with Belonolaimus longicaudatus and P. minor. The inhibiton

of nodulation of soybean by H. glycines was extensive. Ozone and O_3-SO_2 mixture inhibited the growth of soybean both in the presence and absence of nematode. These pollutants also caused cholrosis and necrosis of leaves and increase in the abscission of trifoliate leaves. The effects of pollutant-nematode combinations on nodulation varied because of the severe inhibition of nodulation of soybean by H. glycines. The number of nodules from roots parasitized by this nematode did not differ among the pollutant treatments.

The harmful effect of industrial dust on the nematode, Panagrolaimus rigidus was observed by Kozlowska (1981). Shew et al. (1982) studied the response of tomatoes to ozone, sulphur dioxide and infection by Pratylenchus penetrans. They observed synergistic interaction at 0.2 µl O_3/l, 0.2 µl SO_2/l singly and in combination. However, a mixture of 0.2 ul O_3 and 0.8 µl SO_2/l of air showed antagonistic reaction that caused less change in leaf and shoot dry weight that could be predicted by the main effects of O_3 or SO_2. The presence of P. penetrans attacking the roots enhanced the negative effects of O_3 plus SO_2 on the

leaf growth (dry weight), but suppressed the inhibitory effects of O_3 plus SO_2 on axillary shoot dry weight. Tomato fruit weight was reduced by 0.8 µl SO_2/l of air but the amount of reduction was antagonised by the presence of O_3. Khan (1989) experimented SO_2-O_3 mixture at different concentrations and M. incognita race 1 on tomato and observed that SO_2 and O_3 at 0.2 ppm and Meloidogyne incognita alone significantly reduced plant growth, yield parameters and leaf pigment contents of tomato, Sulphur dioxide (0.2 ppm) and O_3 (0.1, 0.2 ppm) mixture acted synergistcally an caused greater reduction than the sum of their individual effects. Sulphur dioxide and O_3 acted synergistically with the nematode and caused greater reduction in growth and yield parameters than the reductions recorded in nematode inoculated unexposed plants or uninoculated exposed plants. Synergistic reduction in growth of Meloidogyne incognita race 1 inoculated plant exposed to SO_2 plus O_3 at 0.2 plus 0.2 ppm and 0.2 plus 0.1 ppm were also noticed. Fruit count was synergistically reduced by SO_2 plus O_3 at 0.2 ppm each plus nematode.

Ozone (O_3):

The effect of ozone was worked out on tobacco cv. 'Virginia-115' inoculated with Meloidogyne hapla (Bisessar and Palmer, 1984). They observed that ambient O_3 inhibited growth and yield of tobacco regardless of inoculation or non-inoculation with M. hapla. Tobacco inoculated with the nematode suffered more O_3 injury than uninoculated ones. Inoculated plant sprayed with EDU (aethylenediurea) produced 20% less galling than non sprayed ones.

Flyash:

Singh (1989) reported that flyash suppressed the juveniles of root-knot nematode in the ambient and artificial polluted soil. Meloidogyne incognita suppressed more than M. javanica by the application of flyash. The hatching of M. incognita and M. javanica juveniles decreased with the increasing level of flyash mixed in amended soil and these juveniles were inhibited totally at 80 and 90% flyash level.

Singh et al. (1991) found that the population and development of the root-knot nematode decreased with an increase in the concentration of flyash up to 750 t/ha. The availability of N,K and DTPA extractable Cu, Mn, Zn and Fe was more in the nematode inoculated and the flyash amended soils.

Volcanic Ash:

O'Bannon and Santo (1981) conducted an experiment to determine the influence of volcanic ash on the survival and infectivity of root-knot nematode. They reported that ash incorporation had no deliterious effect on root-knot nematode. Different concentrations of 0, 0.5, 1.0, 2.0, 4.0, 8.0, 25, 50 and 100% ash showed no effect to the nematode infection and reproduction on tomato.

Ammonia:

Eno et al. (1955) showed great reduction of plant-parasitic nematode by all levels of anhydrous NH_3 from 136 to 741 ppm. Mojtahedi and Lownsbery (1976) reported that the fertilizer which generated NH_3 was detrimental

to Criconemoides xenoplax. The fertilizer was lethal to nematodes in vitro only when it was accompanied by urease positive bacteria or partially purified urease. The detrimental action of fertilizer- urease mixture was more rapid at pH 8 than pH 7.

Khan (1989) reported that NH_3 at 0.2 ppm caused greater reduction in pre-inoculation exposure. Synergistic interactions between nematode and air pollutants were most consistent in the treatments. In other treatments antagonistic interactions were also noticed in some parameters of tomato. Disease intensity (no. of galls/roots) enhanced in exposed plants. Reproduction of the nematode (no. of eggmass/root) was also inhibited.

Acid Rain:

Shriner (1978) studied the effects of simulated rain acidified with sulphuric acid on host parasite interaction in plant diseases. Reproduction of root-knot nematodes (Meloidogyne hapla) on field-grown kidney beans (Phaseolus vulgaris) exposed to simulated rain of pH 3.2 was inhibited by 66%. Bolla and Huber (1988)

treated pine seedlings with simulating acid rain and inoculated with the White pine specific pathotype of Bursaphelenchus xylophilus VPSt-1. The oleoresin concentration slightly increased while carbohydrate concentration decreased in all seedlings when treated with simulated acid rain (SAR). The changes significantly increased after inoculation of SAR-treated White and Scots pine seedlings with VPSt-1. Wilting was delayed and nematode reproduction decreased in SAR-treated White pine seedlings inoculated with VPSt-1. Simulated acid rain (SAR) treated Austrian pine seedlings were resistant to VPSt-1 but SAR-treated Scots pine seedlings lost tolerance to VPSt-1 and wilted 50-60 days after inoculation.

The influence of simulated acidic rain on interactions of root-knot (Meloidogyne hapla, M. incognita) or cyst (Heterodera glycines) nematodes with soybean plants (Glycine max) was investigated (Shafer et al., 1992). Three days later, plants and soil were exposed to simulated rain to pH 5.3, 4.3, 3.3 or 2.3. After three rains per week for 8 weeks shoot dry weight and production of cyst nematode eggs were

suppressed by 80% and 90% respectively at pH 2.3. Characteristics of polynomial dose — response relationships indicated that the effects of simulated acid rain on plants and nematodes were nematode species dependent. Dose-response relationships for many dependent variables versus rain pH differed between cyst nematodes and root-knot nematodes, but most dose-response characteristics for the two Meloidogyne spp. were similar. Acid deposition can influence nematode plant interactions, but the acidity of simulated rain caused major changes.

INFLUENCE OF SOIL POLLUTANTS ON NEMATODES:

Fresh water resources, though limited in the world, get polluted due to increasing urbanization, industrialization and modern agricultural practices. Waste waters contain objectionable and hazardous constituents which exert direct toxicity or bring about physico-chemical and biological changes in the aquatic environment. Inorganic pollutants like heavy metals, acids, bases and inert substances are hazardous when discharged into water. Free chlorine, chloramines, ammonia, sulphides, even in small concentrations may

cause death to fish. Heavy metal salts such as Cd, Cu, Pb, Hg, Ni, Se, Th, V, Zn and As, etc. are highly toxic to fish, aquatic organisms, animals, microorganisms and man. These metal concentrations range up to 50,000 mg/1. Some of the waste water deteriorate fresh water quality by altering its taste and colour and some others may be detrimental to plants growth and plant yield at their different levels of concentration.

Research work done about the effects of the toxic compounds on soil-inhabiting organisms is still very little compared research of this kind on aquatic organisms. Free-living nematodes are used in toxicological research on environmental pollutants and pesticides by only some research groups (Samoiloff, 1980; Samoiloff et al., 1980; Haight et al., 1982; Mudry et al., 1982; Doelman et al., 1984; Samoiloff and Bogaert, 1984; Coomans and Vanderhaeghen, 1985; Vranken and Help, 1986; Vranken et al., 1985; Frey, 1971; 1976; Neuschulz and Kampfe, 1980; Simpkin and Coles, 1981; Ohba and Ishibashi, 1984). Nematodes have already been used in a bioassay to determine the relative toxic effects of sediments (Howell, 1982; Tietjen and Lee,

1984) or fractions of sediments (Samoiloff et al. 1983).

Bisessar (1982) found higher concentrations of lead, arsenic, cadmium, copper and low population of bacteria, fungi, nematodes and actinomycetes near lead smelter plant. There are several reviews concerning the interaction between pollutants and plant diseases caused by bacteria, fungi and viruses (Manning, 1975; Heagle, 1973; 1982; Huttunen, 1984), however, this type of information is meagre in case of nematode and pollutant interactions (Hodda and Nicholas, 1986; Van Kessel et al., 1989). Few reports are available with respect to the effect of heavy metals and plant-parasitic nematodes.

Van Gundy and Thomason (1962) pointed out that the concentration of 0.5 ppm copper killed Trichodorus christiei within 24h while Hemicycliophora arenaria tolerated concentrations of 4 ppm copper for 48h without apparent injury. Clarke and Shepherd (1965) showed the zinc ions stimulated hatching of seven species of cyst nematodes (Heterodera spp.). Zinc

chloride stimulated more hatching than root diffusate with six species. It was most active at a concentration of 3mM and was still significantly active at 0.2 mM.

Clarke and Shepherd (1966) showed that some inorganic ions inhibited and some promoted hatching to the Heterodera spp. The spontaneous hatch of larvae from eggs in cysts was influenced by the inorganic ions including Ni^{++} in the soil. Toxicity of copper to Trichodorus pachydermus was demonstrated by adding copper salts in known quantities to water. The quick death of the nematodes in copper dishes was striking, while they died more slowly in nickle-plated dishes. Under experimental conditions, viable nematodes were obtained by using nickle-plated supporting sieves sprayed with plastic (Hafkenscheid, 1971). Pitcher and Mc Namara (1972) investigated the toxicity of low concentrations to silver and cupperic ions to three species of plant-parasitic nematodes. In vitro study revealed that Pratylenchus penetrans was most susceptible to silver and Xiphinema diversicaudatum to copper while Aphelencoides ritzemabosi was least affected by either metal.

Haight et al. (1982) obtained Lc50 values for each heavy metal and found Panagrellus to be highly resistant. The chronic effect of seven heavy metals revealed that copper, chromium and cadmium effectively blocked growth at all developmental stages. Nickle and lead at highest soluble concentrations did not affect growth of the worms. The high concentrations of zinc (7500 mg/l) partially blocked growth and mercury was either lethal (20 mg/l) or ineffective in blocking growth (10 ml/1). In another experiment, they studied the effect of heavy metals on the kinetics of pharyngeal pumping in Panagrellus silusiae. They found that as the duration of heavy metal increased, the proportion of feeding worms decreased.

Howell (1982) explained that one probable reason of the survival of nematodes at lower concentrations of the heavy metal may be the secretion of mucus which caused the binding of heavy metal that influenced the intake. Recent work (Howell, 1982a, 1982b,1983, 1984) has indicated that some species of nematodes are very suitable for studies concerning the physiology of accumulation of heavy metals. Howell and Smith (1983)

studied the binding of heavy metals by the marine nematode Enoplus brevis and revealed that the molecular weight of the protiens were estimated to be 29,000 and 63,000 Daltons under denaturing conditions, and under non-denaturing conditions they were estimated to be 28,000 and 450,000 Daltons.

Bisessar et al. (1983) explained the effects of heavy metals and Meloidogyne hapla on celery growth on organic soil near nickle refinery. He observed that soil contaminated with heavy metal increased in the incidence and severity of root-knot disease on celery.

Zinc salts were reported as a stimulant of egg hatching in the soybean cyst nematode, Heterodera glycines (Paul and Leon, 1984). Concentrations of calcium chloride, magnesium chloride and manganese chloride had no effect on hatching of eggs, but reduced hatching at higher concentration by osmotic influences. Hatching of eggs increased as the time of exposure to zinc chloride increased and was maximum at 28^{o}C and pH of 5.3. Picrolonic acid increased Heterodera glycines hatching while sodium metavanadate had no

metal through the marine nematode Enoplus brevis. Cadmium as $CdCl_2$ was first seen in the cuticle. Polyacrylamide gel electrophoresis demonstrated that the metal was bound in the cuticle by a protien with molecular weight (MW) of 450,000 Daltons. Cadmium (^{109}Cd) was detected in the hypodermis and muscle layer where it was associated with protien of MW 28,000 Daltons and finally it accumulated in the gut, associated with a third protien of MW 200,000 Daltons. The meiofaunal nematode populations in mangrove mud-flats adjacent to steel works and chemical factories in the Hunter River Estuary in Australia have been analysed and compared with populations in unpolluted mud-flats nearby. They found that the polluted areas showed more diverse taxonomically, though seasonal variations in population density and other environmental factors complicate the comparison (Hodda and Nicholas 1986). Sturhan (1986, 1988) found that the soil nematode fauna can be affected by heavy metals and other elements; certain nematode taxa may be susceptible and different nematode taxa may react to particular elements in different ways. Nematodes may be suitable as

bioindicators for certain environment chemicals. Zullini and Peretti (1986) reported that the nematode community was sensitive to lead pollution. The total biomass of nematodes, the number of species, and the Shannon diversity index significantly decrease for an increase of the lead content in the moss, while the density of individuals does not seem to be affected by pollution. The nematode suborder Dorylaimina is found to be most sensitive to lead.

Alphey and Brown (1987) studied the effect of pollutants on plant-parasitic nematodes and found that the Xiphinema diversicaudatum and Longidorus elongatus population was significantly decreased by watering with $CuSO_4$ solution and tap water containing 0.5 µg ml^{-1} Cu^{++}. Numbers of trichodorid nematodes were not significantly affected by either of the treatments containing copper. They concluded that small quantities of pollutants adsorbed by the soil particles decreased the toxic effect of these materials upon the soil inhabiting nematodes.

Bolla and Huber (1988) investigated the effect of heavy metals on the susceptibility of pines to pine wood

heavy metals on the susceptibility of pines to pine wood nematode. They found that heavy metal stressed Scots and Austrian pine type VPSt-1. Both the pines infected with heavy-metal-stress and VPSt-1 decreased the concentration of carbohydrate and produced phytotoxic oleoresins. This suggests that the introduction of pinewood nematode, where pines often grow under environmental-stress conditions, may lead to establishment of the nematode and seriously alter forest productivity.

Bolla and Fitzsimmons(1989) studied the effect of copper sulfate and lead acetate on infection of pines with Bursaphelenchus xylophilus and found increase in oleoresin production, stressed energy homeostasis and induced rapid wilting of the seedlings. Scots pine lost tolerance and pine lost resistance to VPSt-1 after the seedlings were treated with either copper sulfate or lead acetate. These results suggests that environmental pollution may significantly affect susceptibility of pines to B. xylophilus and may have a role in establishment of this nematode in uninfested areas. The studies on saprophytic nematode Caenorhabditis elegans,

various concentrations of $CdCl_2$ revealed that the growth and reproduction of the organisms was significantly reduced from a level of 1 µM $CdCl_2$. At levels of 160 and 320 µM, growth was retarded at the early juvenile stages and the organisms did not reach the adult stage and could therefore not reproduce (Van Kessel et al., 1989)

Weiss and Larink (1991) studied the influence of sewage sludge and heavy metals on nematodes in an arable soil and reported that total nematode numbers were highest in the site treated with sewage sludge and heavy metals but in control site it was found in small quantity. The plant-parasitic nematode genera showed different patterns of abundance depending on the sludge treatment and heavy metals content. For the mycophagic and bacteriophagic nematodes number increased with the amount of sludge especially in the sites with a higher heavy metal content. The family Rhabditae was the most numerous group in the sludge plus heavy metal treatment. In contrast to these findings the omnivorous nematodes were very rare in the sludge treated plots and were completely absent in plots treated with sludge

plus heavy metals, whereas predatory nematodes were numerous only after the application of sludge alone.

PLAN OF WORK

It is well known that plant-parasitic nematodes have specific relationships with their host plants. There are chances of interference of this relationship by soil pollutants. However, there is paucity of information of this aspect. Therefore, it was considered desirable to investigate the effect of soil pollutants on plant-parasitic nematodes, the details of which are as follows:

1. To study the effect of industrial wastes from an electroplating plant on the larval hatching of the root-knot nematode, Meloidogyne incognita in vitro.

2. To study the effect of industrial wastes from an an electroplating plant on the mortality of the second stage juvenile (J_2) of the root-knot nematode, Meloidogyne incognita in vitro.

3. To study the effect of industrial wastes from an electroplating plant on the larval penetration of root-knot nematode Meloidogyne incognita into the roots of tomato (Lycopersicon esculentum) cv. Pusa Ruby.

4. To study the effect of industrial wastes from an electroplating plant on the root-knot development caused by the root-knot nematode Meloidogyne incognita and plant growth of tomato (Lycopersicon esculentum) cv. Pusa Ruby.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Preparation and sterilization of soil:

Sandy loam soil which was thoroughly mixed with river sand and organic manure in the ratio of 3:1:1 and sieved through 16 mesh sieve was used throughout the coarse of investigation. Fifteen cm earthen pots were filled with this soil mixture at the rate of 1 kg/pot. The pots were then sterilized in an autoclave at 20 lb pressure maintained for 20 minutes. The sterilized pots were allowed to cool at room temperature before being used for experiments.

3.2 Raising and maintenance of test plant:

Seeds of the test plant tomato, Lycopersicon esculentum cv. Pusa Ruby were surface sterilized with 0.1% solution of mercuric chloride for two minutes and rinsed in sterilized water several times, and then sown in sterilized pots. Seedlings so obtained were used for carrying out the experiment throughout the course of study.

3.3 Raising and maintenance of pure culture of root-knot nematode:

The pure culture of the root-knot nematode, Meloidogyne incognita was raised on tomato plants using single eggmass collected from heavily infected tomato roots. The eggmass was sterilized by treating it with 1:5 aqueous solution of chlorex (calcium hypochlorite) for 5 minutes as described by Den Ouden (1958). The treated eggmass was allowed to hatch on a coarse sieve lined with double layered tissue paper at 27^{o}C under aseptic conditions. The sieve was placed in a petriplate containing so much water that only the brim of the sieve touches it. Tomato seedlings grown in 30 cm earthen pots containing sterilized soil were inoculated with second stage juveniles so obtained. Nematode eggmasses from these plants were then used for multiplying the culture. For the identification and verification of species, a slide of perineal pattern of the same female from which the first eggmass was picked, was prepared and examined under microscope.

3.4 Preparation of nematode inoculum:

For root-knot nematode, large number of healthy eggmasses were picked-up with the aid of sterilized forcep and needle from the roots of heavily infected tomato, on which the pure culture was maintained. These eggmasses were washed with distilled water and were placed over a sieve having double layered tissue paper for hatching. The sieve was placed over a petridish containing water, the level of which was adjusted that it just touches the brim of the sieve. For obtaining large number of second stage juveniles for inoculation, a series of such assembles were made. After 24 hour the nematode suspension was collected from the petridishes in a beaker and fresh water was added to the petridishes.

Water suspension of the nematode was thoroughly stirred to obtain a homogenous nematode distribution before taking 5 ml suspension in a counting dish (Doncaster, 1962) for counting the number of nematodes under the stereoscopic microscope. An average of 3 counts was made to determine the nematode density in

the suspension. Volume of suspension having nematodes was such adjusted that each ml contained 1000 nematodes. This was obtained by either adding more water depending upon the situation, so that 5 ml suspension may be poured into each pot to provide the required amount of inoculum level.

3.5 **Experiment procedure**:

Effluents containing chromium (Cr) and nickle (Ni) were obtained from an electroplating plant, Santry Flash Light, Industrial Estate and Sigma Engineering Works, Anupshar Road, Aligarh. The procedures adopted for different experiments are as follows:

***Experiment No. 1*:To study the effect of industrial wastes from an electroplating plant on the larval hatching of the root-knot nematode, *Meloidogyne incognita* in vitro.**

Five freshly picked eggmasses of average size were transferred to petridishes (40 mm diameter) containing 5 ml solutions of different concentrations of the effluents containing heavy metals. All the treatments were replicated three times including distilled water as control. The petridishes were incubated at 25^{o}C and

the total number of hatched juveniles were counted after 5 days, under stereoscopic microscope with the help of counting dish (Doncaster, 1962).

***Experiment No. 2*:To study the effect of industrial wastes from an electroplating plant on the mortality of second stage juveniles (J_2) of the root-knot nematode, Meloidogyne incognita in vitro.**

About 100 freshly hatched second stage juveniles (J_2) of the nematode were transferred to petridishes (40 mm diameter) containing 5 ml solution of different concentrations of the effluents following the procedure described by Alam (1985). Each treatment including distilled water control was replicated three times. The total number of immobile larvae were counted after 12, 24, 36 and 48 hour under stereoscopic microscope. Death of the nematodes was ascertained by transferring them into plain water and then percent mortality was determined.

***Experiment No. 3*:To study the effect of industrial wastes from an electroplating plant on the larval penetration of the root-knot nematode, Meloidogyne incognita into the roots of tomato, Lycopersicon esculentum cv. Pusa Ruby:**

For this experiment river sand was washed with plenty of water so that all the dust particles were

removed. The sand was then filled in icecream cups at the rate of 50g sand per cup. Roots of three-week-old seedlings of tomato cv. Pusa Ruby were dipped in 1,2 and 3% effluent simultaneously for 12, 24, 36 and 48 h. Then these seedlings were transplanted at one seedling/cup. In control set the tomato seedlings were dipped in distilled water in place of the effluents. Next day 1000 freshly hatched second stage juveniles of Meloidogyne incognita were inoculated to each plant. One week after inoculation, seedlings were gently uprooted and washed with water to remove sand particles. The juveniles were isolated from the sand by using Cobb's sieving and decanting method (Southey, 1986). The suspension so obtained was taken in a beaker and 3 ml of this suspension was transferred to counting dish (Doncaster, 1962). The number of nematodes left after penetration were counted in each cup under stereoscopic microscope. This number was deducted from the initial number used for the inoculation and thus number of larvae which had penetrated into the roots were determined.

***Experiment No.4*: To study the effect of industrial wastes from an electroplating plant on the root-knot development caused by the root-knot nematode, *Meloidogyne incognita* and plant growth of tomato, *Lycopersicon esculentum* cv. Pusa Ruby:**

Three-week-old tomato seedlings were transplanted in 15 cm clay pots, containing 1 kg of autoclaved soil. The pots were treated with different doses of the effluents, e.g. 0.01, 0.02 and 0.03 ml/pot and later inoculated with second stage juveniles of the root-knot nematode at 5000 J_2/pot. Untreated pots served as control. Each treatment was replicated three times. The experiment was terminated after three months and plant growth characters and other parameters were assessed.

3.6 Recording of the data:

Plants were uprooted after 60 days of inoculation and the root system was thoroughly washed under running tap water. Excess amount of water was removed by putting the roots and shoots between blotting papers. The length (cm), fresh and dry weight (g) of roots and shoots were determined separately. Plant length was recorded from the tip of the first leaf to the longest root. For recording the dry weight, shoots and roots were dried in an oven at 60°C for 48h.

Root-knot index (RKI) was rated on 0-5 scale of Taylor and Sasser (Sasser et al., 1984) as given below:

0 = No galls

1 = 1-2 galls/root system

2 = 3-10 galls/root system

3 = 11-30 galls/root system

4 = 31-100 galls/root system

5 = >100 galls/root system

3.7 Statistical analysis:

Statistical analysis of the data for critical difference (C.D.) at P=0.05 and P=0.01 was done as per procedure described by Pansey and Sukhatme (1978).

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Soil pollution due to heavy metals like Hg, Pb, Co, Cr, etc. has been well recognized throughout the world. Refineries, metal smelters, caustic soda factories, paper mills, power plant discharges, soap factories, fertilizer industries, electroplating units, etc. are mainly responsible for causing heavy metal pollution.

Soil waste dumps and residues of mining, ore processing and smelting operations are common sources of higher local concentrations of heavy metals in the ground water (Matthess, 1972; 1974; Schottler, 1972).

Industrial effluents, domestic waste materials and sewage water are discharged into land and rivers and also reach the crop fields. They may be absorbed by roots and thus toxic substances accumulate in different parts of the plant (Westing, 1969).

The effluents and wastes either directly injure the roots when come in contact or indirectly impair the

growth of plants due to accumulation in different parts. Adverse effects of the industrial effluents containing heavy metals on the crops such as bean, pea, wheat, tomato, etc. have been demonstrated by many workers (Ajmal et al., 1984, Ajmal and Khan, 1984).

Several reports of effects of pollutants on plant pathogens have been published (Heagle, 1973), but relatively few have concerned nematodes. In greenhouse bioassay (Temple and Bisessar, 1981), root-knot nematode , Meloidogyne hapla retarded growth of lettuce leaves by 50% in metal contaminated soil and 30% in control soil.

In the present study an attempt has been made to evaluate the toxicity of effluents from an electroplating plant containing two heavy metals, viz. chromium and nickle, to the root-knot nematode, Meloidogyne incognita (Kofoid & White) Chitwood and the disease caused by it on tomato, Lycopersicon esculentum (L.) Karsten. The results of different experiments are discussed here under.

4.1 To study the effect of industrial wastes from an electroplating plant on the larval hatching of the root-knot nematode, *Meloidogyne incognita in vitro*.

Hatching of juveniles of the root-knot nematode, *Meloidogyne incognita* was found to be greatly influenced by the effluents containing heavy metals, viz. chromium (Cr) and nickle (Ni). Chromium had more inhibitory effects than nickle (Table 1).

In chromium containing effluent percent inhibition in the larval hatching of *M. incognita* over control was 90.41, 80.51 and 65.15% at 3,2 and 1 % concentrations respectively (Table 1, Fig.1).

Nickle containing effluent also significantly inhibited the larval hatching of *M. incognita* at all the higher concentrations, i.e. 2 and 3% but the lowest concentration (1%) showed the least toxicity. The percent inhibition in the hatching over control was 57.71, 49.99 and 40.36% in 3, 2 and 1% concentrations of nickle containing effluent respectively (Table 1,Fig.2).

Table 1. Influence of different effluents containing heavy metals on the juveniles hatching of the root-knot nematode, *Meloidogyne incognita in vitro.*

Treatment	Concentration(%)	*Number of J_2 hatched per eggmass (5 days)	Percent inhibition in hatching over control
Chromium(Cr)	DW	665	--
	1	225	65.15
	2	126	80.51
	3	62	90.41
C.D. (P=0.05)		069.84	
C.D.(P=0.01)		105.81	
Nickle (Ni)	DW	665	--
	1	386	40.36
	2	327	49.39
	3	274	57.70
C.D. (P=0.05)		094.99	
C.D. (P=0.01)		143.90	

Each value is an average of three replicates
* Round off values.

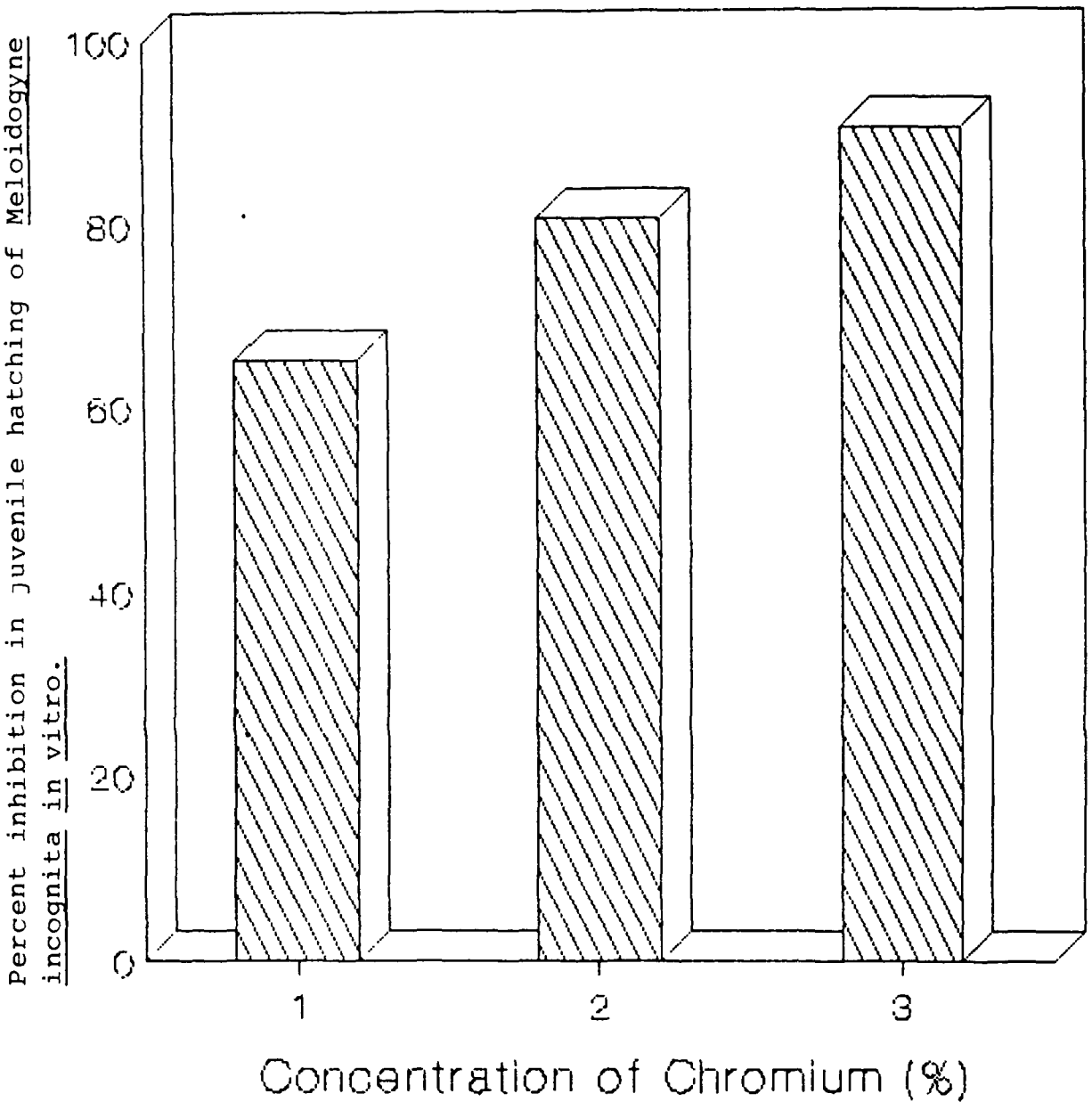

Fig.1. Effect of effluent containing chromium on the hatching of the root-knot nematode, Meloidogyne incognita in vitro.

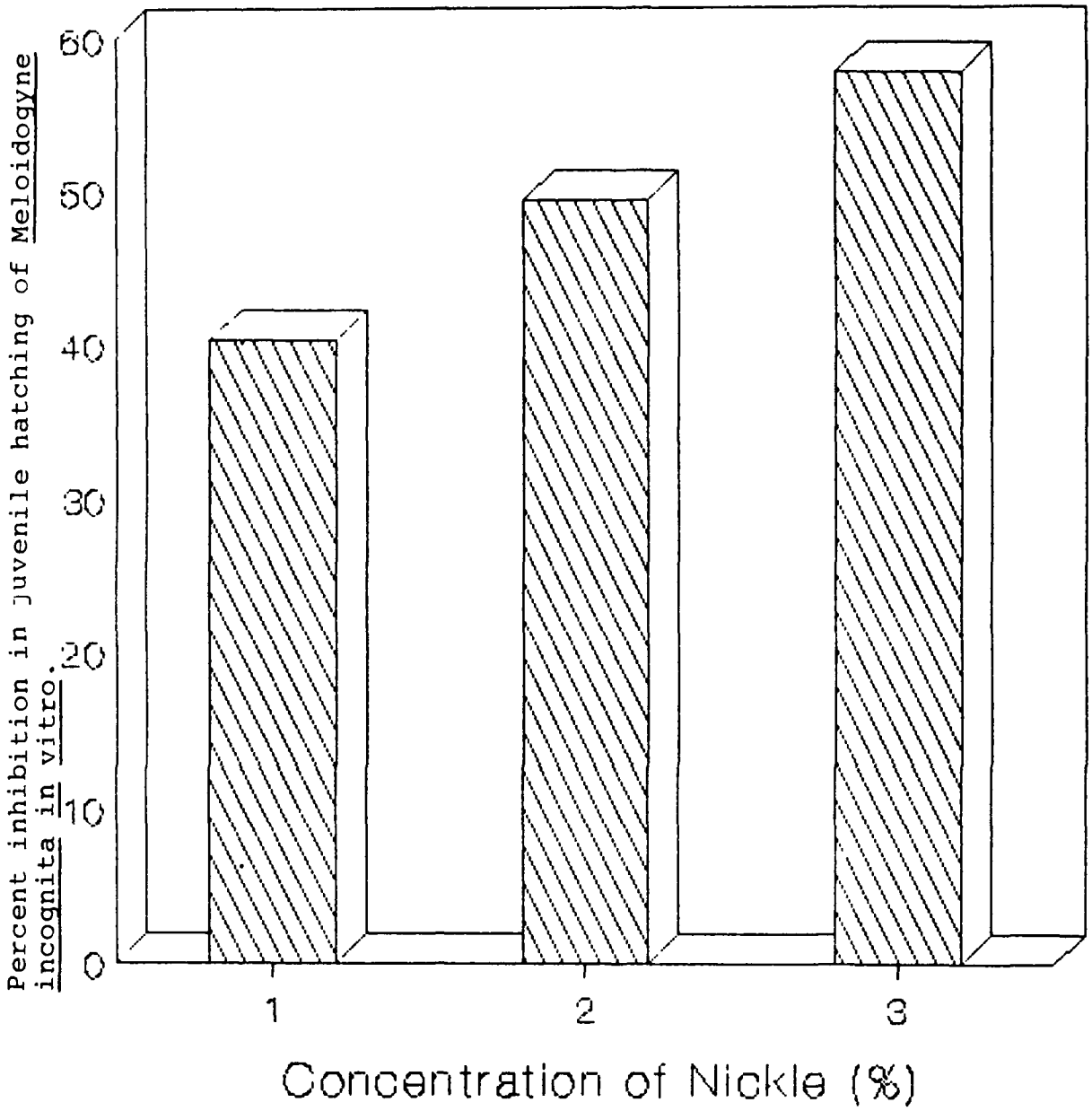

Fig.2. Effect of effluent containing nickle on the hatching of the root-knot nematode, Meloidogyne incognita in vitro.

4.2 To study the effect of industrial wastes from an electroplating plant on the mortality of second stage juveniles (J_2) of the root-knot nematode, Meloidogyne incognita in vitro.

Results of in vitro studies revealed that mortality of second stage juveniles (J_2) of the root-knot nematode, Meloidogyne incognita increased with an increase in the exposure period and concentration of the effluents containing heavy metals, e.g. chromium and nickle. Chromium was found to be more toxic among the two effluents.

Juvenile mortality of M. incognita was observed in chromium containing effluent to be 45.0,50.0 and 60.0% at 12h, 55.0, 66.0 and 81.0% at 24h, 70.0, 88.0 and 96.0% at 36h and 76.0, 91.0 and 100.0% at 48h exposure period at 1,2 and 3% concentrations respectively (Table.2). Absolute mortality was recorded at 3% concentration at 48h exposure period (Table.2).

Nickle containing effluent showed comparatively less toxicity. In this effluent the mortality of second stage juvenile (J_2) M.incognita increased with an increase in the concentration and the exposure period. Mortality of juveniles was found to be 15.8, 19.0 and

Table 2. Influence of different effluents containing heavy metals on the mortality of second stage juveniles (J_2) of the root-knot nematode, *Meloidogyne incognita in vitro.*

Treatment	Exposure period(h)	Percent mortality in different conc. of the effluent				Regression Value
		DW	1%	2%	3%	
Chromium (Cr)	12	0 (11 0)	45 0 (29 5)	50 0 (47 9)	60 0 (66 4)	$\hat{Y}$=38 75+18 4(X-1 5)
	24	10 0 (19 4)	55 0 (41 8)	66 0 (64 2)	81 0 (86 6)	$\hat{Y}$=53 00+22 4(X-1 5)
	36	14 0 (29 9)	70 0 (54 6)	88 0 (80 3)	96 0 (104 0)	$\hat{Y}$=67 00+24 7(X-1 5)
	48	16 0 (30 7)	76 0 (57 4)	91 0 (84 1)	100 0 (110 0)	$\hat{Y}$=70 70+26 7(X-1 5)
Nickle (Ni)	12	0 (4 0)	15 8 (11 0)	19 0 (18 1)	23 1 (25 3)	$\hat{Y}$=14 50+07 2(X-1 5)
	24	10 0 (13 0)	24 0 (20 1)	28 0 (27 4)	35 0 (34 7)	$\hat{Y}$=23 70+07 3(X-1 5)
	36	14 0 (19 0)	32 0 (26 0)	35 0 (33 6)	38 0 (41 0)	$\hat{Y}$=29 70+07 8 (X-1 5)
	48	16 0 (20 8)	36 0 (29 6)	40 0 (38 4)	44 0 (47 2)	$\hat{Y}$=34 00+08 8(X-1 5)

Each value is an average of three replicates.
In parentheses are given calculated values.

23.1% at 12h exposure period, 24.0, 28.0 and 35.0% at 24h, 32.0, 35.0 and 38.0% at 36h and 36.0 40.0 and 44.0% at 48h exposure period at 1,2 and 3% concentrations of nickle respectively (Table.2).

Linear relationship was found between the concentration of the effluent containing heavy metals and mortality (Fig. 3). Chromium at 1% concentration showed more than 50% mortality after 36h which increased to 100.0% after the exposure period of 48h. At 12h exposure period 3% concentration also caused more than 50% mortality of M. incognita juveniles (Table.2).

The present study also revealed Ec 50 value of the effluent containing heavy metals for the root-knot nematode, M. incognita. With the help of regression line Ec 50 vlaue for chromium was calculated to be 2.15% at 12h exposure, 1.40% at 24h, 0.85% at 36h and 0.75% at 48h exposure period (Fig.3).

According to Khan et al. (1986) none of the juveniles of M. incognita survived in the test concentrations of mercury (100, 1000, 2500, 5000 and 8000 ppm) except in 100 ppm where some of the juveniles survived as their mortality was found to be 92.75%.

Van Gundy and Thomason (1962) in the experiment observed the quick death of the nematodes in copper dishes while they died more slowly in nickle-plated

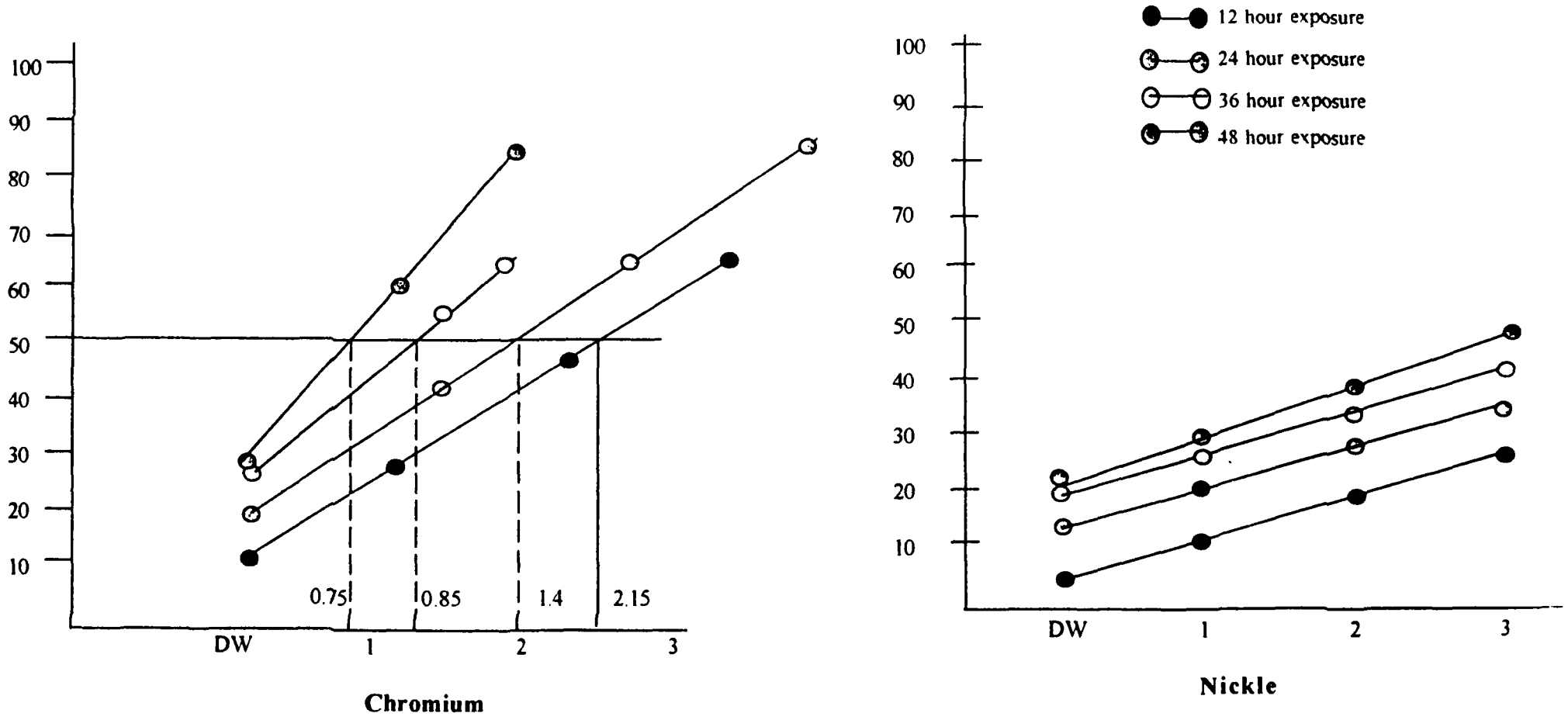

Fig.3. Regression lines showing linear relationship between different concentrations of the effluents containing heavy metals (Cr and Ni) and percent mortality of second stage Juveniles of the root-knot nematode, *Meloidogyne incognita in vitro*.

dishes. Copper ions, released from incomplete nickle-plating, could have caused this, in co-operation with Ni^{++} ions.

Ec 50 value for plant-parasitic nematodes has not yet been studied so far. The present study shows quite revealing information regarding the Ec 50 values of the effluent containing heavy metals for the plant-parasitic nematodes. Ec 50 value for chromium and nickle was calculated with the help of regression line. The Ec 50 values for chromium was observed to be 2.15% (12h), 1.4% (24h), 0.85% (36h) and 0.75% (48h) while nickle showed less than 50% mortality of J_2 at all the exposure periods thus showed less toxicity than chromium (Fig.3).

4.3 To study the effect of industrial wastes from an electroplating plant on the larval penetration of root-knot nematode, Meloidogyne incognita into the roots of tomato, Lycopersicon esculentum cv. Pusa Ruby.

The effluents containing chromium and nickle were found to be inhibitory to larval penetration of Meloidogyne incognita into the roots of tomato. The inhibition in larval penetration increased with an increase in the concentration of the effluent as well as the duration of root-dips in the effluents. Chromium was found to be more inhibitory than nickle for the root penetration of the second stage juvenile (J_2) of

the root-knot nematode, Meloidogyne incognita.

The minimum number of nematodes which penetrated into the roots of tomato was 53.0 after 48h root-dip in 3% concentration of the effluent containing chromium while the maximum number was observed as 74.0 after 12h root-dip at 1% concentration of the effluent as compared to 93.0 in un-dipped control (Table 3, Fig. 4).

Effluent containing nickle also had an inhibitory effect on the penetration of second stage juveniles (J_2) of the nematode at different concentrations, viz. 1,2 and 3% of the effluent for different dip durations, e.g. 12, 24, 36 and 48h. The minimum number of juveniles which could penetrate into the roots was 80.00 when roots were dipped in 3% effluent for 48h, while the maximum number was recorded to be 86.0 after 12h dip duration in 1% of the effluent as compared to 93.0 in control (Table 4, Fig. 5).

It may be possible that the root-dip treatment in the effluents brought about absorption and accumulation of some toxic substances including chromium and nickle rendering it unsuitable for larval penetration.

Table 3. Effect of bare root-dip treatment in different concentrations of effluent containing chromium (Cr) on the larval penetration of the root-knot nematode *Meloidogyne incognita* into the roots of tomato *Lycopersicon esculentum* cv. Pusa Ruby.

Root-dip duration (h)	Conc.of effluent (%)	No. of J_2 penetrated per plant	Percent reduction over control
12	-	88	--
	1	74	15.8
	2	73	17.9
	3	69	21.4
	C.D.(P=0.05)	7.45	
	C.D.(P=0.01)	11.29	
24	--	89	--
	1	72	20.4
	2	68	24.4
	3	67	25.8
	C.D. (P=0.05)	4.27	
	C.D. ((P=0.01)	6.46	
36	--	92	--
	1	69	24.4
	2	67	27.1
	3	66	28.2
	C.D. (P=0.05)	3.51	
	C.D. (P=0.01)	5.33	
48	-	93	--
	1	60	34.4
	2	57	38.6
	3	53	43.2
	C.D. (P=0.05)	3.21	
	C.D. (P=0.01)	4.86	

Each value is an average of three replicates.

* Round off values.

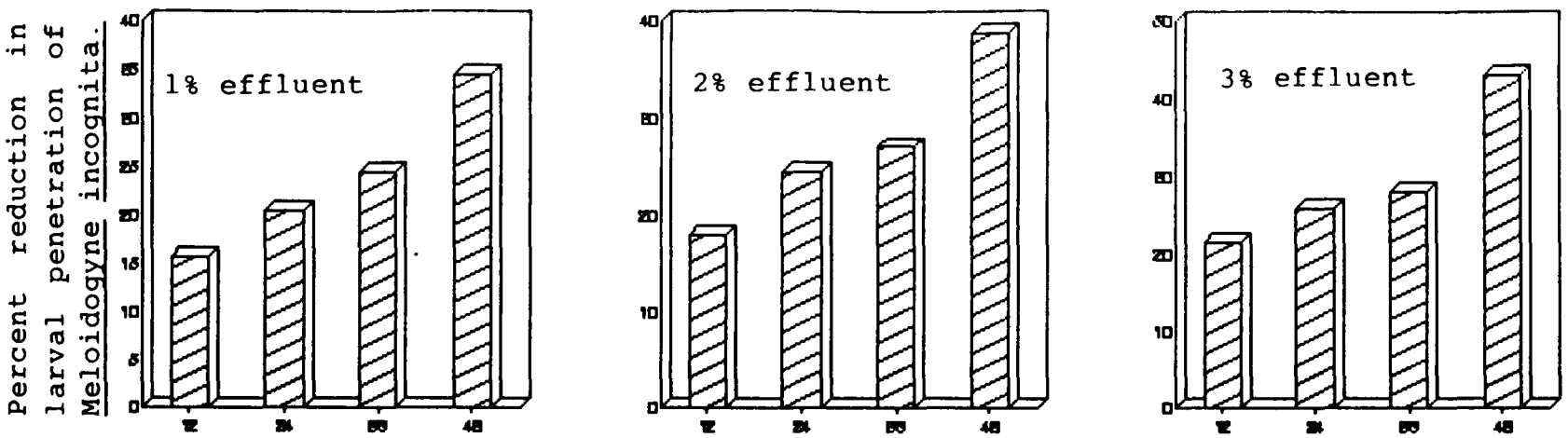

Fig.4. Effect of bare root-dip treatment in different concentrations of effluent containing chromium (Cr) on the larval penetration of the root-knot nematode Meloidogyne incognita into the roots of tomato, Lycopersicon esculentum cv. Pusa Ruby.

Table 4. Effect of bare root-dip treatment in different concentrations of effluent containing nickle (Ni) on the larval penetration of the root-knot nematode *Melidogyne incognita* into the roots of tomato *Lycopersicon esculentum* cv. Pusa Ruby.

Root-dip duration (h)	Conc.of effluent (%)	No. of J_2 penetrated per plant*	Percent reduction over control
12	-	88	--
	1	86	2.4
	2	85	3.3
	3	83	5.7
	C.D.(P=0.05)	3.89	
	C.D.(P=0.01)	5.89	
24	--	90	--
	1	85	5.7
	2	84	7.0
	3	82	8.7
	C.D. (P=0.05)	1.45	
	C.D. ((P=0.01)	2.20	
36	--	92	--
	1	84	8.4
	2	83	9.8
	3	81	11.4
	C.D. (P=0.05)	1.43	
	C.D. (P=0.01)	2.18	
48	-	93	--
	1	83	10.8
	2	81	12.2
	3	80	14.0
	C.D. (P=0.05)	0.51	
	C.D. (P=0.01)	0.78	

Each value is an average of three replicates.

* Round off values.

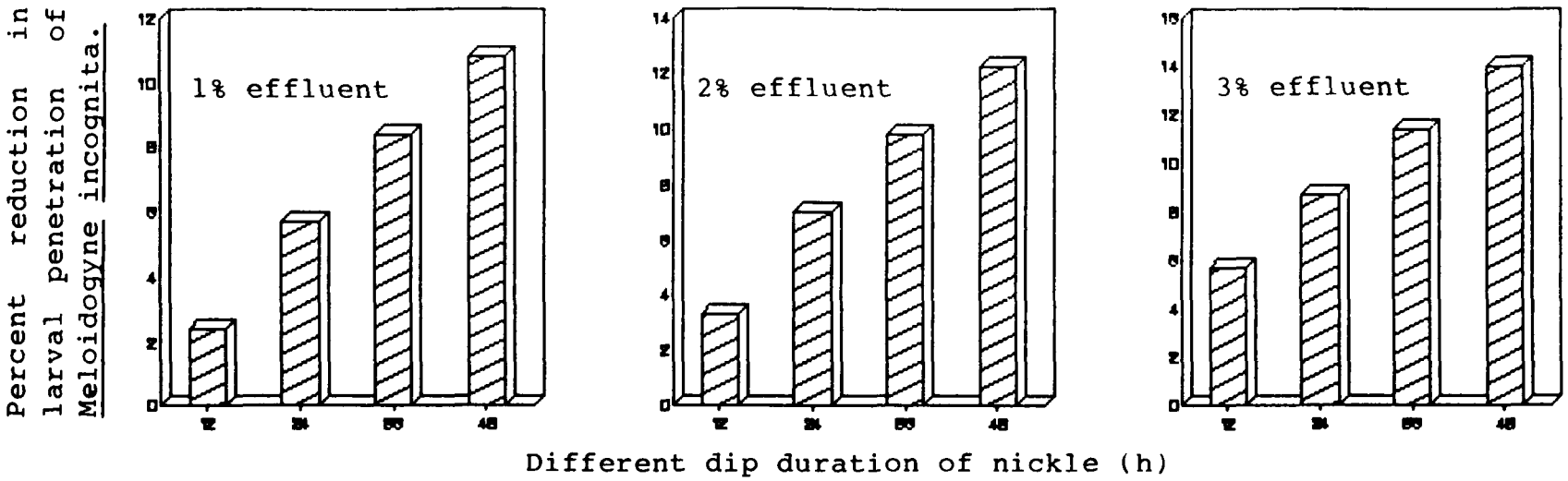

Fig.5. Effect of bare root-dip treatment in different concentrations of effluent containing nickle (Ni) on the larval penetration of the root-knot nematode Meloidogyne incognita into the roots of tomato, Lycopersicon esculentum cv. Pusa Ruby.

4.4 To study the effect of industrial wastes from an electroplating plant on the root-knot development caused by root-knot nematode Meloidogyne incognita and plant growth of tomato Lycopersicon esculentum cv. Pusa Ruby.

Plant growth of tomato cv. Pusa Ruby decreased as the concentration of the effluent containing chromium increased. Plant length was recorded to be 80.26 cm in untreated and uninoculated (control) and 66.43 cm in untreated and inoculated set. In the treated and uninoculated set plant length was 69.66, 58.66 and 55.00 cm showing percent reduction to the tune of 13.20, 26.91 and 31.47% in single, double and triple strength whereas in the treated and inoculated set plant length was 60.92, 56.16 and 48.00 cm and percent reduction 24.09, 30.02 and 40.19% respectively for single, double and triple strength of the effluent containing chromium over control (Table 5).

There was reduction in fresh weight of tomato plants from 40.53 to 21.56 g when plants were inoculated with Meloidogyne incognita (J_2). In the treated (Cr) and uninoculated set fresh plant weight was 31.86, 29.10 and 23.40 g showing percent reduction of 21.39%, 28.20% and 42.26% respectively for single,

Table 5. Influence of different concentrations of the effluent containing chromium on root-knot development caused by root-knot nematode *Meloidogyne incognita* and plant growth of tomato *Lycopersicon esculentum* cv. Pusa Ruby.

Treatment	Length(cm)			Fresh Weight (g)			Dry Weight (g)			Root-knot index (0-5 scale)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	
Untreated UN	55 00	25 26	80 26	29 06	11 46	40 53	6 63	2 50	9 13	
Untreated IN	45 00	21 43	66 43 (17 23)	15 00	6 53	21 56 (46 80)	2 43	1 10	3 53 (61 33)	3 00
Treated (SS) UN	47 50	22 16	69 66 (13 20)	23 13	8 73	31 86 (21 39)	3 20	1 33	4 53 (50 38)	
Treated (SS) IN	39 66	21 26	60 92 (24 09)	13 36	5 00	18 36 (54 70)	2 33	0 66	3 00 (67 14)	2 33 (22 33)
Treated (DS) UN	38 33	20 33	58 66 (26 91)	21 20	7 90	29 10 (28 20)	2 96	1 00	3 96 (56 62)	
Treated (DS) IN	36 33	19 33	56 16 (30 02)	11 30	4 26	15 56 (61 60)	2 00	0 50	2 50 (72 61)	2 00 (33 33)
Treated (TS) UN	37 33	17 66	55 00 (31 43)	16 63	6 73	23 40 (42 26)	1 90	0 73	2 63 (17 19)	
Treated (TS) IN	31 16	16 66	48 00 (40 19)	9 83	3 53	13 36 (67 03)	1 53	0 33	1 86 (79 62)	1 66 (44 66)
C D (P=0 05)	2 53	2 50		1 30	1 10		0 41	0 51		0 74
C D (P=0 01)	3 50	3 40		1 80	1 53		0 58	0 21		1 12

Each value is an average of three replicates.
In parentheses are given percent reduction over untreated-uninoculated control.
UN=Uninoculated, IN=Inoculated with 5000 *Meloidogyne incognita* (J_2) per plant.
SS=Single strength (0.01 ml effluent per kg soil).
DS=Double strength (0.02 ml effluent per kg soil).
TS=Triple strength (0.03 effluent per kg soil).

double and triple strength of the effluent over untreated and uninoculated control (Table 5, Fig. 6). In the treated and inoculated set fresh plant weight was 18.36, 15.56 and 13.36 g and percent reduction 54.70, 61.60 and 67.03% respectively for single, double and triple strength of the effluent containing Cr over control (Table 5, Fig. 6).

As a result of inoculation with the root-knot nematode, M. incognita dry weight of tomato plants significantly reduced from 9.13 to 3.53 g in control plants without any treatment (Fig. 7). Treatment with the effluent containing chromium caused significant reduction in dry weight of tomato plants both in presence and absence of the root-knot nematode (Fig. 7). Highest reduction in dry weight was observed in plants treated with the triple strength of the effluent and inoculated with the nematode (79.62%) followed by 72.61 and 67.14% at double and single strength of the effluent containing chromium over control (Table 5) while in the treated and uninoculated set, dry weight was reduced by 71.19%, 56.62% and 50.38% at different concentration (triple, double and single) of chromimum respectively (Table 5).

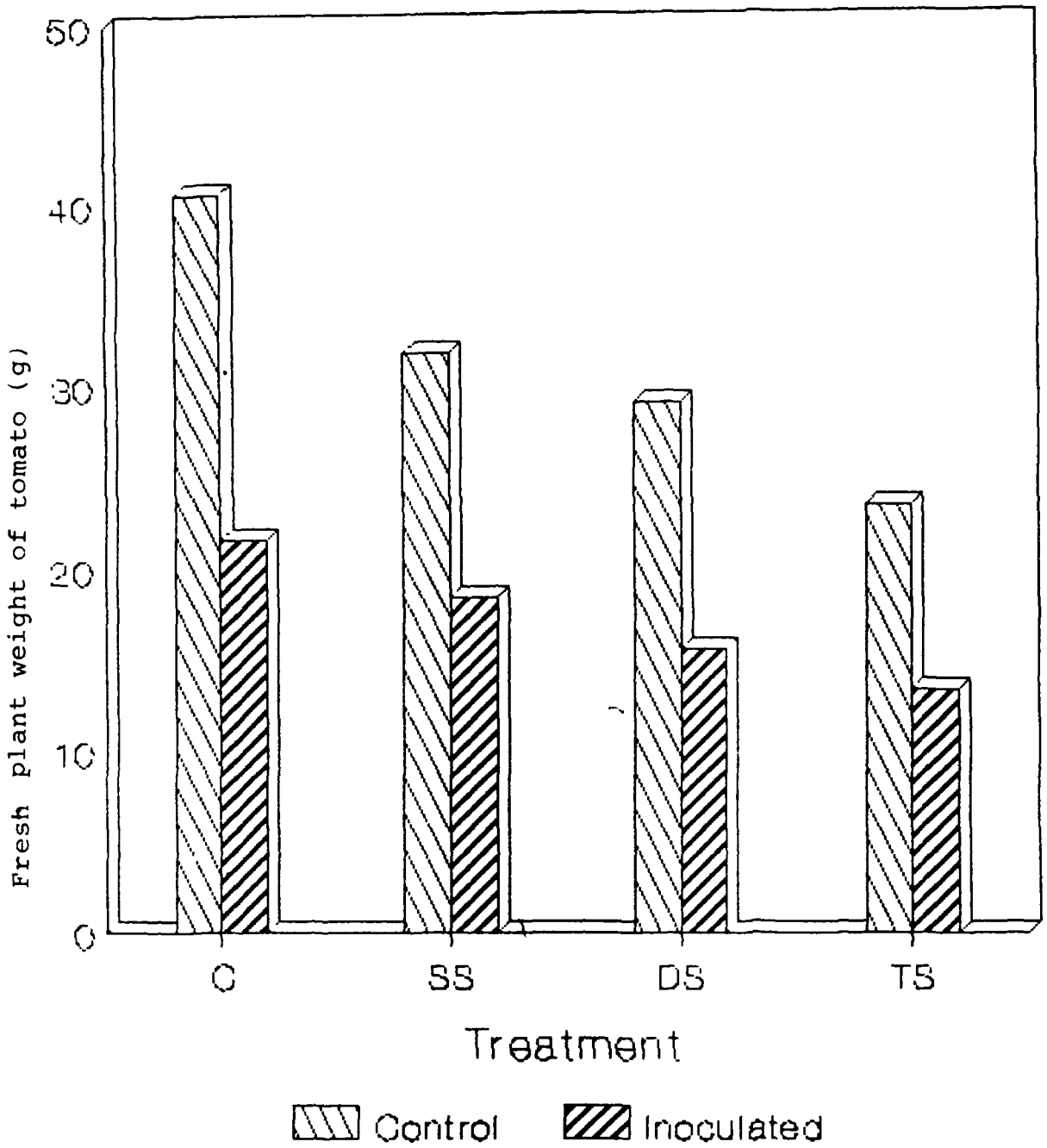

Fig.6. Influence of different concentrations of the effluent containing chromium on the fresh weight of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil, DS=Double strength, 0.02 ml effluent per kg soil; TS=Triple strength, 0.03 ml effluent per kg soil, Inoculum level= 5000 J_2 of Meloidogyne incognita per plant).

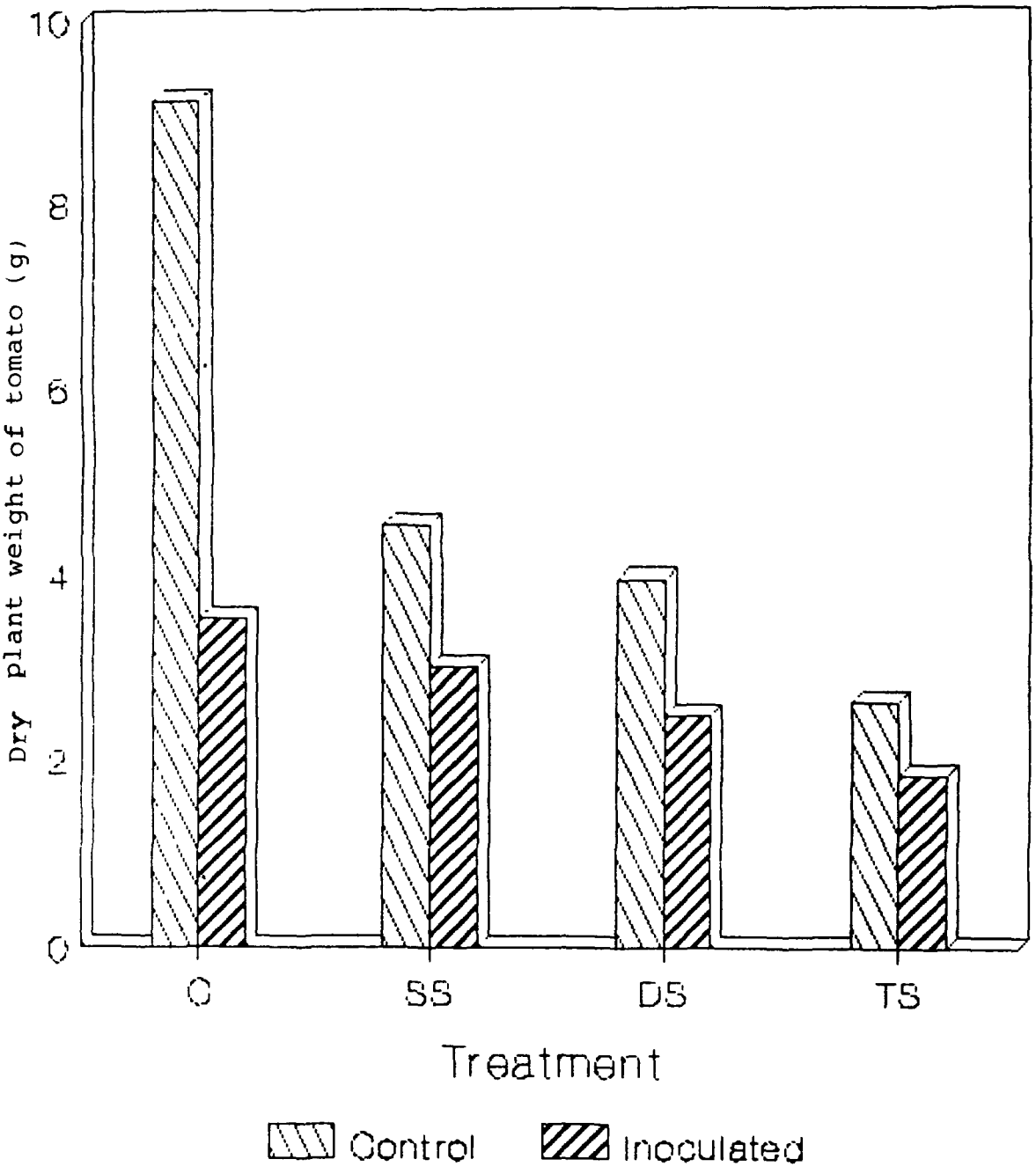

Fig.7. Influence of different concentrations of the effluent containing chromium on the dry weight of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil; DS=Double strength, 0.02 ml effluent per kg soil; TS=Triple strength, 0.03 ml effluent per kg soil; Inoculum level=5000 J_2 of Meloidogyne incognita per plant).

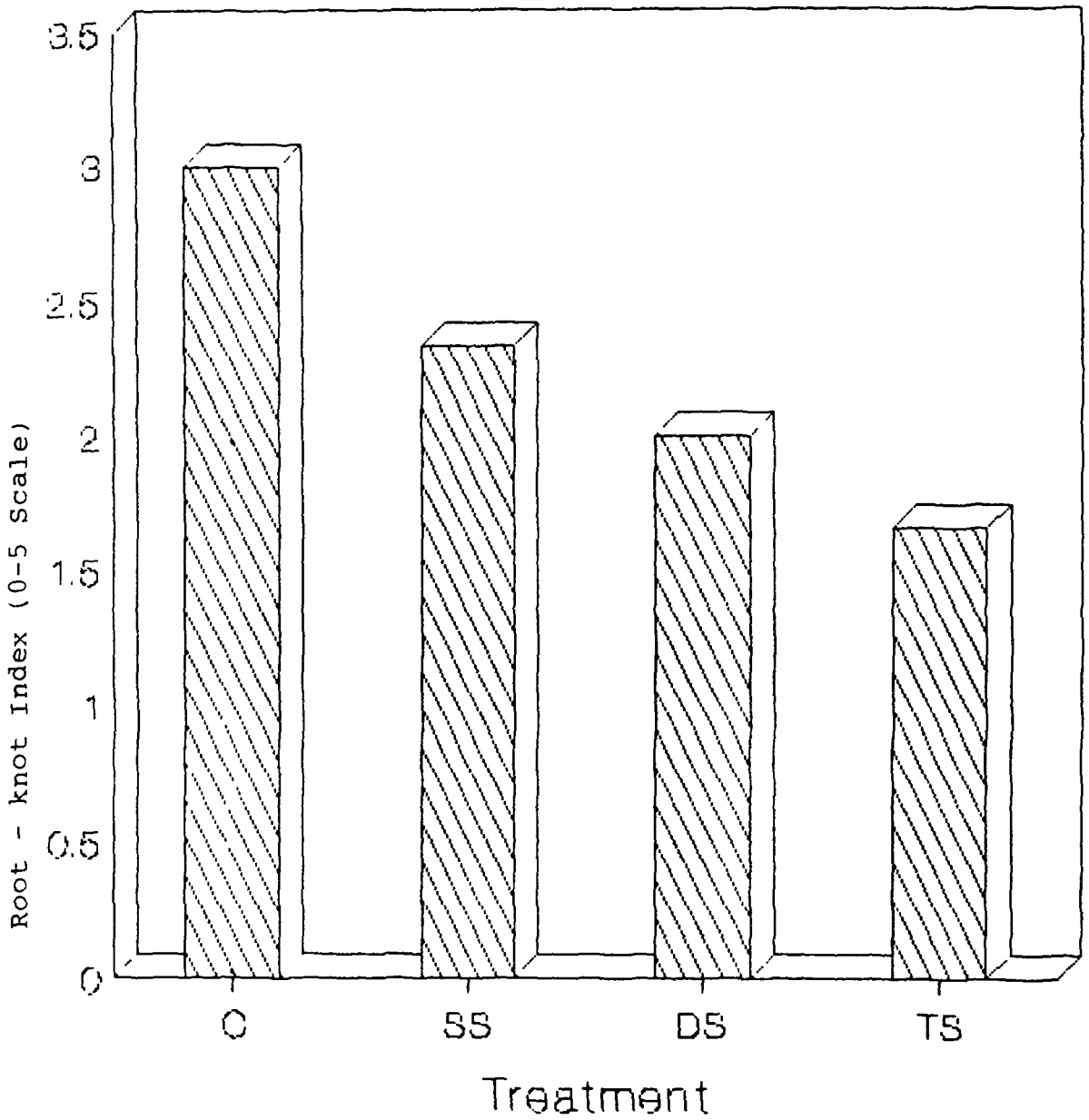

Fig.8. Influence of different concentrations of the effluent containing chromium on the root-knot development of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil; DS=Double strength, 0.02 ml effluent per kg soil; TS=Triple strength, 0.03 ml effluent per kg soil; Inoculum level=5000 J_2 of Meloidogyne incognita per plant).

Gall counts in tomato roots from the effluent treated and nematode inoculated set were significantly lower than in roots from untreated control. Therefore, it may be concluded that heavy metals contamination of the soil resulted in decrease in the incidence of root-knot disease in tomato. Thus, growth of tomato was severely retarded by the effluent containing chromium and the added stress of Meloidogyne incognita caused further growth inhibition (Table 5, Fig. 8).

In case of the effluent containing nickle plant length of tomato cv. Pusa Ruby decreased with an increase in the concentration of the effluent. This effluent was however found to be less toxic as compared to that containing chromium. Length of tomato plants reduced from 80.26 cm to 66.43 cm when plants were inoculated with Meloidogyne incognita. In the effluent (Ni) treated and inoculated set plant length reduced to 51.90, 48.36 and 41.90 cm respectively in triple, double and single strength of the effluent. Corresponding figures for the effluents treated and uninoculated set were 70.03, 57.43 and 49.86 cm respectively (Table 6). Highest percent reduction in plant length of tomato was

recorded to be 47.79, 39.74 and 35.33% in the effluent treated and nematode inoculated set respectively at triple, double and single strength of the effluent containing nickle. In the effluent treated and uninoculated set percent reduction was observed to be 37.87, 28.44 and 12.74% at the above concentrations, viz. triple, double and single strength of the effluent containing nickle respectively (Table 6).

When tomato plants were inoculated with the root-knot nematode Meloidogyne incognita, fresh plant weight reduced from 40.56 g to 21.56 g (Table 6, Fig. 9). In the effluent (Ni) treated and nematode inoculated set percent reduction in fresh weight increased with an increase in the concentration of the effluent containing nickle, it was found to be 55.76%, 51.81% and 30.52% respectively with triple, double and single strength of the effluent. In the same way, in the effluent treated and uninoculated set percent reduction of fresh weight of the plant was recorded to be 40.78, 39.02 and 20.40% at different concentrations, viz. triple, double and single strength of the effluent containing nickle over control respectively (Table 6, Fig. 9).

Table 6. Influence of different concentrations of the effluent containing nickle on root-knot development caused by root-knot nematode *Meloidogyne incognita* and plant growth of tomato *Lycopersicon esculentum* cv. Pusa Ruby.

Treatment	Length(cm)			Fresh Weight (g)			Dry Weight (g)			Root-knot index (0-5 scale)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	
Untreated UN	55 00	25 26	80 26	29 06	11 46	40 53	6 63	2 50	9 13	
Untreated IN	45 00	21 43	66 43 (17 23)	15 00	6 56	21 53 (46 80)	2 43	1 10	3 53 (61 33)	3 00
Treated (SS) UN	50 80	19 20	70 03 (12 74)	22 73	9 60	32 26 (20 40)	4 63	1 60	6 33 (31 76)	
Treated (SS) IN	34 60	17 56	51 90 (35 33)	20 76	7 40	28 16 (30 52)	3 50	1 26	4 76 (47 86)	2 60 (11 33)
Treated (DS) UN	41 00	16 43	57 43 (28 44)	19 76	8 60	28 36 (39 02)	2 63	1 36	4 00 (56 18)	
Treated (DS) IN	32 80	15 58	48 36 (39 74)	14 46	5 06	19 53 (51 81)	2 16	1 16	3 33 (63 52)	2 30 (22 33)
Treated (TS) UN	36 33	13 53	49 86 (37 87)	17 80	6 53	24 00 (40 78)	2 06	1 03	3 10 (66 04)	
Treated (TS) IN	25 90	12 33	41 90 (47 79)	13 00	4 93	17 93 (55 76)	1 20	0 92	2 10 (76 99)	2 00 (33 33)
C D (P=0 05)	3 97	1 34		1 79	0 87		0 47	0 27		1 37
C D (P=0 01)	5 51	1 86		2 49	1 20		0 65	0 37		2 08

Each value is an average of three replicates.
In parentheses are given percent reduction over untreated-uninoculated control.
UN=Uninoculated, IN=Inoculated with 5000 *Meloidogyne incognita* (J_2) per plant.
SS=Single strength (0.01 ml effluent per kg soil).
DS=Double strength (0.02 ml effluent per kg soil).
TS=Triple strength (0.03 ml effluent per kg soil).

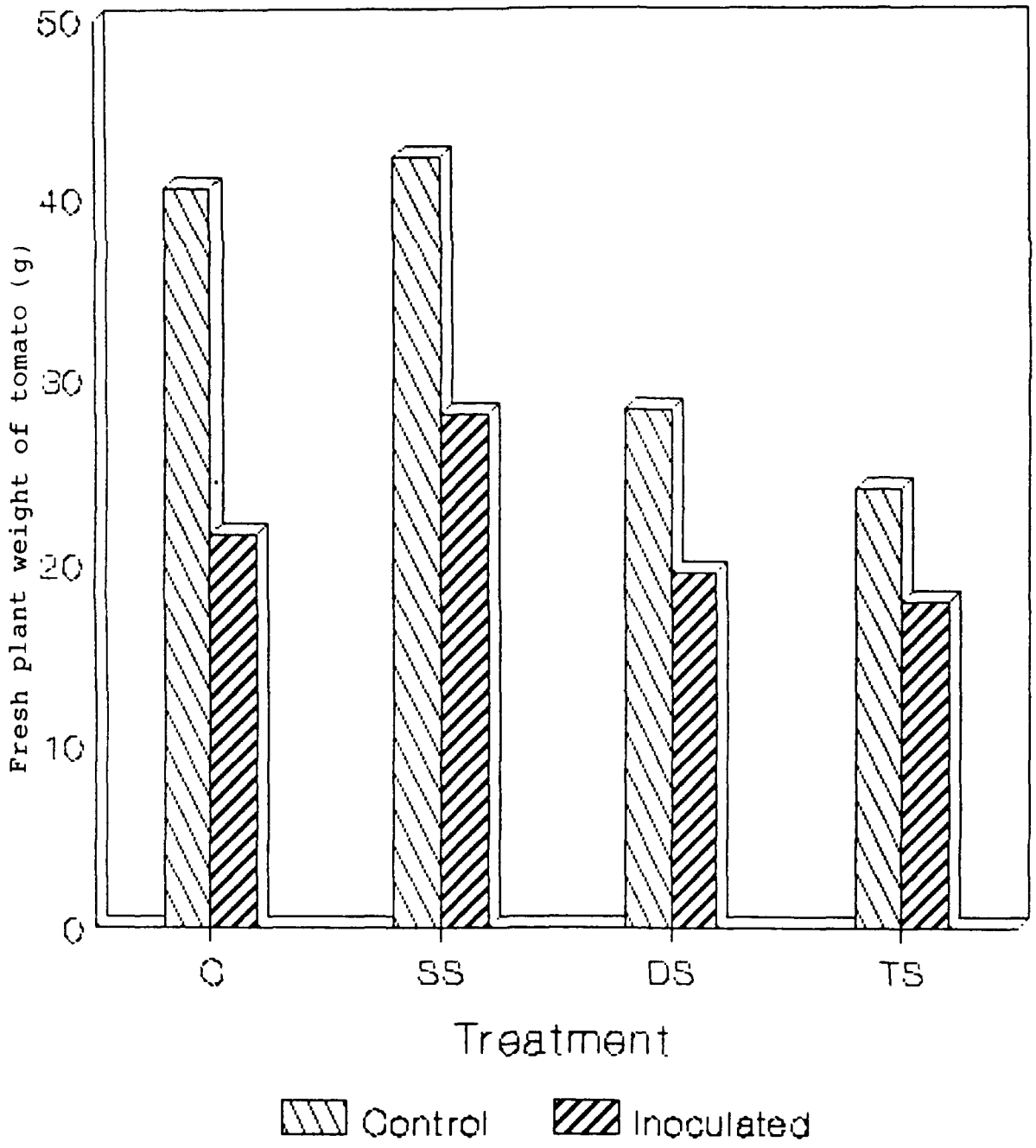

Fig.9 Influence of different concentrations of the effluent containing nickle on the fresh weight of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil; DS=Double strength, 0.02 ml effluent per kg soil; TS= Triple strength, 0.03 ml effluent per kg soil; Inoculum level=5000 J_2 of Meloidogyne incognita per plant).

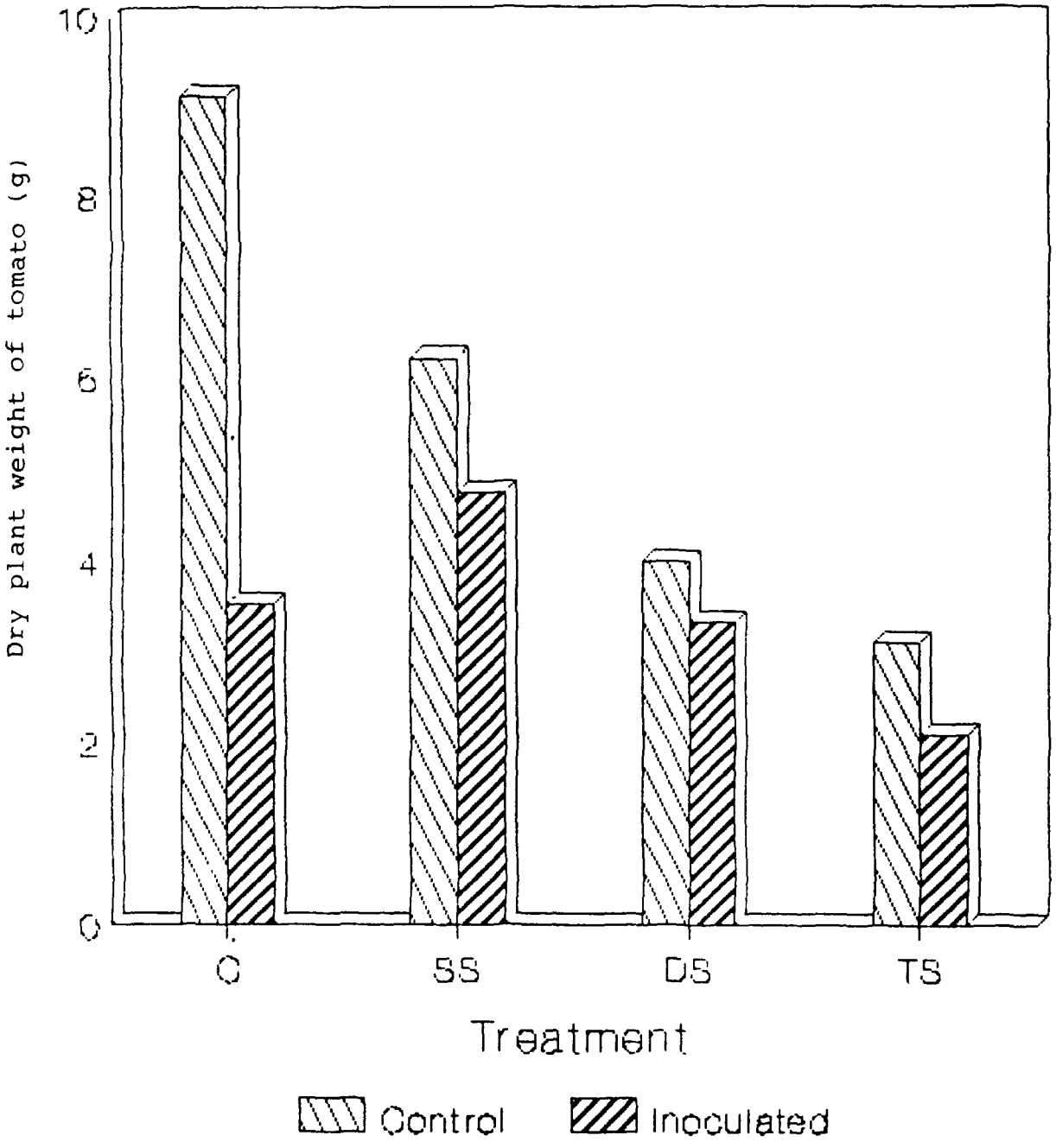

Fig.10. Influence of different concentrations of the effluent containing nickle on the dry weight of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil; DS=Double strength, 0.02 ml effluent per kg soil; TS=Triple strength, 0.03 ml effluent per kg soil; Inoculum level=5000 J_2 of Meloidogyne incognita per plant).

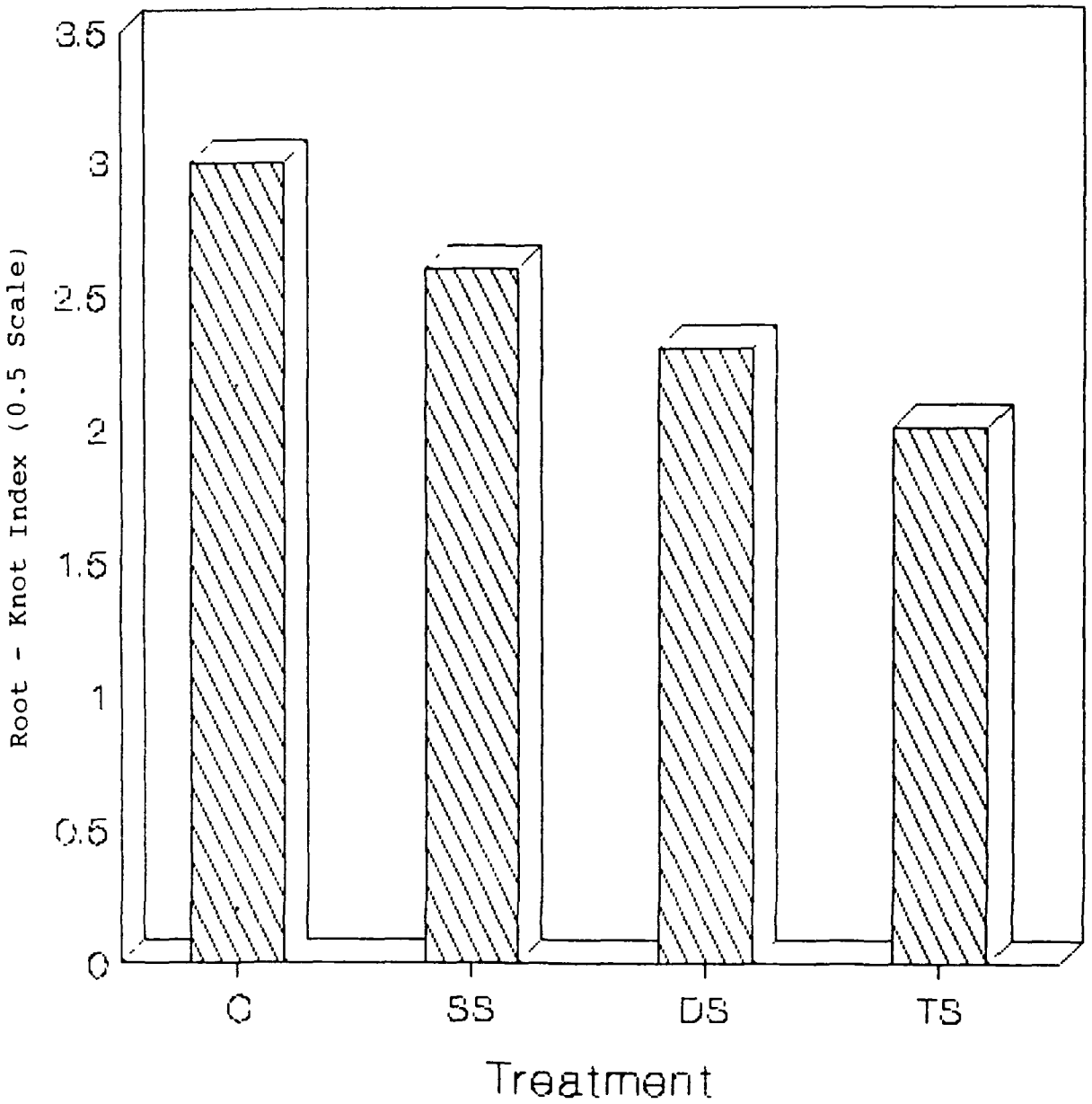

Fig.11. Influence of different concentrations of the effluent containing nickle on the root-knot development of tomato, Lycopersicon esculentum cv. Pusa Ruby. (C=Untreated control; SS=Single strength, 0.01 ml effluent per kg soil; DS=Double strength, 0.02 ml effluent per kg soil; TS=Triple strength, 0.03 ml effluent per kg soil; Inoculum level=5000 J_2 of Meloidogyne incognita per plant).

Percent reduction of dry weight of tomato plants also increased with an increase in the concentration of the effluent containing nickle. It was recorded to be 76.99, 63.52 and 47.86% in the effluent (Ni) treated (triple, double and single strength) respectively and inoculated set. In the effluent (Ni) treated and uninoculated set percent reduction in dry weight of tomato plant increased; figures for reduction were 66.04, 56.18 and 31.76% respectively at triple, double and single strength of the effluent containing nickle over control (Table 6, Fig. 10).

Number of galls on tomato roots in the effluent (Ni) treated and inoculated set were significantly less than that of untreated and inoculated set. Therefore, it may be concluded that soil contamination with the effluent containing nickle caused decrease in root-knot incidence on tomato (Table 6, Fig. 11).

Poor root galling in effluent treated plants appears to be due to toxic effect of the effluents, and inhibition in the larval penetration into the roots and their subsequent development.

SUMMARY

5. SUMMARY

An attempt has been made to evaluate the effect of effluents of electroplating plant containing chromium and nickle on larval hatching, mortality and root penetration of second stage juveniles of Meloidogyne incognita and subsequent root-knot development tomato, Lycopersicon esculentum cv. Pusa Ruby. Summary of the results is given below.

1. Both the effluents were found to be highly inhibitory to the larval hatching of the root-knot nematode, Meloidogyne incognita in vitro. Chromium was found to be more inhibitory to larval hatching than nickle. Range of inhibition of larval hatching was 65.15 to 90.41% in chromium containing effluent and 40.36 to 57.70% in nickle containing effluent in the test doses of 1-3% of the effluent.

2. Both the effluents were found to be highly toxic to second stage juveniles (J_2) of Meloidogyne

incognita in vitro. Chromium containing effluent was more toxic than nickle containing effluent. The mortality of second stage juvenile increased with an increase in the concentration of the effluents (1-3%) and duration of exposure (12-48h). Ec 50 value was also determined with the help of regression line. For chromium containing effluent, Ec 50 values was 2.15, 1.40, 0.85 and 0.75%. In all the test concentrations of nickle containing effluent mortality was less than 50% in all the exposure period tested.

3. Both the test effluents containing chromium and nickle significantly inhibited larval penetration into the roots of tomato Lycopersicon esculentum. Chromium containing effluent caused greater inhibition in larval penetration than nickle containing effluent. The minimum number of juveniles which could penetrate into the root system of tomato was 5.30 after 48h dip-duration in 3% concentration of the effluent containing chromium while maximum number was found as 74.0 after 12h dip-duration at 1% concentration of the

effluent containing chromium as compared to 93.0 in the un-dipped control. In case of the other effluent containing nickle the minimum number of second stage juveniles (J_2) which could permeate into the roots was 80.00 when roots were dipped in 3% effluent for 48h, while maximum number was recorded to be 86.0 after 12h dip-duration in 1% concentration of the effluent as compared to 93.0 in the un-dipped control.

4. Results of experiment clearly revealed that both the effluents significantly inhibited growth parameters (length, fresh/dry weights) of plants of tomato Lycopersicon esculentum cv. Pusa Ruby. As expected chromium containing effluent was found to be more injurious to plant growth than nickle containing effluent. There was further inhibition in plant growth parameter when plants were also inoculated with the root-knot nematode Meloidogyne incognita. Both the effluents also suppressed root-knot development. After inoculation with Meloidogyne incognita, there was reduction in fresh weight of plants from 40.53 to

21.56 g and in the treated (Cr) and uninoculated set fresh plant weight was 31.86, 29.10 and 23.40 g showing percent reduction of 21.39, 28.20 and 42.26% respectively for single, double and triple strength of the effluent over untreated and uninoculated control. In the treated and inoculated set fresh plant weight was 18.36, 15.56 and 13.36 g. There was increase in the percent reduction of plant in the treated and uninoculated set. In such sets, the percent reduction in fresh weight was found as 54.70, 61.60 and 67.03% respectively for single, double and triple strength of the effluent containing chromium over control. The same trend was observed in the reduction of dry weight of tomato plants. Root-knot index (RKI) was highest (RKI 3.00) in untreated control. Highest reduction in root-knot index was observed in plants treated with the triple strength of the effluent and inoculated with the nematode (RKI 1.66) followed by RKI 2.00 and RKI 2.33 at double and single strength of the effluent containing chromium over control.

In case of nickle fresh plant weights were 40.56 and 21.56 in uninoculated and inoculated sets respectively. In the treated (Ni) and uninoculated set fresh weight of the tomato plant was recorded as 32.26, 28.36 and 24.00 g with percent reduction in fresh weight 20.40, 39.02 and 40.78% for single, double and triple strength of the effluent containing nickle respectively. In the treated and inoculated set, reduction in fresh plant weight was also noted which was 28.16, 19.53 and 17.93 g respectively showing 30.52, 51.81 and 55.76% percent reduction for single, double and triple strength of the effluent respectively over untreated and inoculated control. Dry weight also reduced from 9.13 to 3.53 g due to the nematode inoculation. In the treated and uninoculated set, dry weight was observed to be 6.23, 4.00 and 3.10g and percent reduction 31.76, 56.18 and 66.04% for single, double and triple strength of the effluent containing nickle. Likewise, same trend was observed in treated and inoculated set. Dry weight decreased as the strength of the effluent increased and reduction in dry weight was found to be 4.76, 3.33 and 2.10 g and

percent reduction 47.86, 63.52 and 76.99% at above concentrations of the effluent viz. single, double and triple strength of the effluent containing nickle respectively. The root-knot index (RKI) was rated as 2.60 over control with the single strength of the effluent containing nickle and RKI 2.30 and RKI 2.00 with double and triple strength of the effluent against RKI 3.00 in control.

REFERENCES

6. REFERENCES

Ajmal, M. and Khan, A.U., (1984). Effects of brewery effluent on agricultural soil and crop plants. *Environ. Pollut.* (Series A) **33**: 341-351.

Ajmal, M.; Khan, M.A. and Nomani, A.A. (1984). Effect of industrial dairy processing on soil and crop plants. *Environ.Pollut.* **33**: 97-106.

Alam, M.M. (1985). A simple method for *in vitro* screening of chemicals for nema-toxicity. *Int. Nematol. Network Newsl.* (U.S.A.). **2**(1): 6.

Alphey, T.J.W. and Brown, D.J.F. (1987). Effect of pollutants on plant-parasitic nematodes. *Test Agrochem. Cult.* ***8*** *(Ann. Appl. Biol.* 110 Supplement): 38-39.

Anon (1971). Estimated crop losses due to plant-parasitic nematodes in the United States. *Sppl. J. Nematol. Special Publ.* No: 1, 7 pp.

Antonio, H. (1988). Avalicao das, perdas causadas for *Meloidogyne incognita* raea a no cultivar BR 4 desoja. *Nematologica Brasileira* **12**: 29-34.

Backett, P.H.T. and Davis, K.I. (1978). The additivity of the toxic effects of copper, nickle and zinc in young barley. *New Phytol.* **81**: 155-173.

Bassus, W. (1968). On the effects of industrial emissions on the population of nematodes in the soil of pine forests. *Pedobiologia* **8**: 289-295.

Bisessar, S. (1982). Effect of heavy metals on micro-organisms in soil near a secondary lead smelter. Water, *Air and Soil Pollut.* **17**: 305-308.

Bisessar, S. and Palmer, K.T. (1984). Ozone antioxidant spray and Meloidogyne hapla effects on tobacco (Nicotiana tabacum) cv. Virginia-115. *Atmos. Environ.* **18**: 1025-1028.

Bisessar, S.; Rinne, R.J. and Potter, J.W. (1983). Effects of heavy metals and Meloidogyne hapla on celery grown on organic soil near a nickle refinery. *Plant Dis.* **67**(1): 11-14.

Bolla, R.I. and Fitzsimmons K. (1988). Effect of simulated acid rain on Bursaphelenchus xylophilus infection of pine seedlings. *J. of Nematology* **20**(4): 590-598.

Bolla, R.I. and Huber, M.C. (1988). Heavy metal effects on pine susceptibility to pinewood nematode. *J. of Nematology* **20**(4): 629-630.

Clarke, A.J. and Shepherd, A.M. (1965). Zinc and other metallic ions as hatching agents for the beet cyst nematode Heterodera schachtii Schm. *Nature Lond.* **208**:502.

Clarke, A.J. and Shepherd, A.M. (1966). Inorganic ions and the hatching of Heterodera spp. *Ann. Appl.Biol.* **58**: 497-508.

Coomans, A. and Vanderhaeghen, R. (1985). In vitro testing toxicities. Exercise 29. *In Plant Nematology Laboratory Manual*(B.M. Zuckermann, W.F. Mai, and M.B. Harrison, Eds.), pp. 172-176, University of Massachusetts Agricultural Experiment Station, Amherst. M.A.

Doelman, P.; Nieboer, G.; Schrooten, J.; and Visser, M. (1984). Antagonistic and synergistic toxic effects of lead and cadmium in a simple food chain: Nematode feeding on bacteria and fungi. *Bull. Environ. Contam. Toxicol.* **32** : 717-723.

Doncaster, C.C. (1962). A counting dish for nematodes. *Nematologica* **7**: 334-336.

Dropkin, V.H. (1980). Introduction to Plant Nematology. New York: John Wiley and Sons, 298 pp.

Eno, C.F; Blue, W.G. and Good, J.M. (1955). The effect of anhydrous ammonia on nematode, fungi, bacteria and nitrification in some Florida soil. *Proceedings Soil Science Society of America* **19**: 55-58.

Epstein, E. and Chaney, R.L. (1978). " Land disposal of toxic substances and water related problems." *J. Wat. Pollut. Cont. Fed.***6**: 518 -522.

Feldmesser, J.; Edwards, D.I.; Epps, J. M.; Held, C.M.; Jenkins, W.R.; Jonsen, H.J.; Lear, B.; McBeth, C.W.; Nighi, E.L. and Perry, V.G. (1971).

Estimated crop losses due to plant-parasitic nematodes in the United States. *Supp. J. Nematol.* Special publication No.1. Report of Soc. Nematologists Committee on Crop losses, 1970.

Frank, R.; Ishida, K. and Suda, P. (1976). Metals in agricultural soils of Ontario. *Can. J. Soil Sci.* **56**: 181-196.

Frey, F. (1971). Über die Eignung von *Acrobeloides buetschlii* (Cephalobidae) für nematologische Laboruntersuchungen. *Nematologica* **17**: 474-477.

Frey, F. (1976). Untersuchungen über die wirkung von im obstbau verwendeten Herbiziden auf den Testnematoden *Acrobeloides buetschlii* (de Man 1884) Steiner and Buhrer 1933. Z. *Pflanzenkrankh Phlansenschutz.* **83**: 434-441.

Froster, W.A. (1954). Toxic effects of heavy metals on crop plants grown in soil culture. *Ann. Appl. Biol.* **41**: 637-651.

Goodbold, D.L. and Hutterman , A. (1985). Effect of zinc, cadmium and mercury on root elongation of

Picea abies (Karst.), seedling and the significance of these metals to forest die-back. *Environ. Pollut.* **38**: 375-381.

Gotoh, S., Tokudome, S. and Koga, H. (1979). Mercury in soil derived from igneous rock in northern Kyushu, Japan. *Soil Sci.* and *Plant Nutr.* **24**: 391-406.

Graft, H.J. and Schwantes, H.O. (1983). Der rinflu Bvon Cadmium, Zinc, Beluind Quecksilbe aufdie Atmung und Garung von *Saccharomyces cerevisiae*. *Angew. Bot.* **57**: 31-43.

Hafkenscheid, H.H.M. (1971). Influence of Cu^{++} ions on *Trichodorus pachydermus* and an extraction method to obtain active specimens. *Nematologica* **17**: 535-541.

Haghiri, F. (1973). Cadmium uptake by plants. *J. Environ. Qual.* **2**: 93-96.

Haight, M.; Mudry, T. and Pasternak, J. (1982). Toxicity of seven heavy metals on *Panagrellus silusiae:* The efficacy of the free-living nematode as an *in vivo* toxicological bioassay. *Nematologica* **28**:1-11.

Hale, J.C.; Ormrod, P.J.; Laffey, P.J. and Allen, O. (1985). Effect of nickle and copper mixtures on tomato in sand culture. *Environ. Pollut.* **39**: 53-59.

Hassett, J.J.; Miller, J.E. and Koeppe, D.E. (1976). Interaction of lead and cadmium by roots. *Environ. Pollut.* **11**: 297-302.

Heagle, A.S. (1973). Interaction between air pollutants and plant parasites. *Ann. Rev. Phytopathology* **11**: 365-388.

Heagle , A.S. (1982). Interaction between air pollutants and parasitic plant disease. *In: Effects of gaseous air pollutants in agriculture.* M.H. Unsworth and (D.P. Ormrod, eds.) pp. 333-334. Butterworth Scientific, London.

Heck, W.W.; Heagle, A.S. and Shriner, D.S. (1986). Effects on vegetation: Native, Crops, forests. Pages 247-350, In : *Air Pollution*, volume **6**.A.S. Stern, ed. Academic Press, New York.

Heggested, H.E. (1988). Reduction of soybean seed yields by ozone air pollution. *J. Air Pollut. Control. Assoc.* **38**: 1040-1041.

Hodda, M. and Nicholas, W.L. (1986). Nematodes diversity and industrial pollution in the hunter river estuary, NSW, Australia. *Marine Pollut. Bull.* **17**(6): 254-255.

Howell, R. (1982). The secretion of mucus by marine nematode (*Enoplus* spp.): A possible mechanism influencing the uptake and loss of heavy metals pollutants *Nematologica* **28**: 110-114.

Howell, R. (1982a). The incidence of heavy metal pollutants in *Enoplus brevis* (Bastian) and *Enoplus communis* (Bastian) with some observations on toxicity accumulation, depuration and metal binding by proteins. *Nematologica* **28**: 110-114.

Howell, R. (1982b). Levels of heavy metal pollutants in two species of marine nematodes. *Mar. Pollut. Bull.* **13**: 396-398.

Howell, R. (1983). Heavy metals in marine nematodes: Uptake, tissue distribution and loss of copper and zinc. *Mar. Pollut. Bull.* **14**: 263-268.

Howell, R. (1984). Acute toxicity of heavy metals to two species of marine nematodes. *Mar. Environ. Res.* **11**: 153-161.

Howell, R. and Smith, L. (1983). Binding of heavy metals by the marine nematode *Enoplus brevis* Bastian, 1865 . *Nematologica* **29**: 39-48.

Howell, R. and Smith, L. (1985). Flux of cadmium through the marine nematode *Enoplus brevis* Bastian, 1865. *Revue De Nematologie* **8**(1): 45-51.

Hunter, J.G. and Vergnano, O. (1952). Nickle toxicity in plants. *Ann. Appl. Biol.* **39**: 279-284.

Hutchinson, M.T.; Reed, J.P.; Streu, H.T.; Edward, A.A. and Schroeder, P.H. (1961). Plant parasitic nematodes of New Jersey. *New Jersey Agric. Stat. Rutsers Bull.*, 796.

Hutchinson, T.C. and Whitby, L.M. (1974). Heavy metal pollution in the sudbury mining and smelting region of Canada I: Soil and vegetation contamination by nickle, copper and other metals. *Environ. Conserv.* **1**: 123-132.

Huttunen, S. (1984). Interactions of disease and other stress factors with atmospheric pollution. pp 346-390 in M. Treshow, ed. *Air pollution and plant life*. New York: John Wiley and Sons.

Katsuhiro, I. and Shigenori, A. (1969). Retention of mercury by soil colloids. *Soil Sci. and Plant Nutr.* **15**: 86-91.

Katznelson, H. and Henderson, V.E. (1963). Ammonium as an attractant for a soil nematode. *Nature Lond.* **198**: 907.

Khan, M.R. (1989). Studies on root-knot nematodes in relation to environmental pollution on vegetable crops. *Ph.D. Thesis, A.M.U., Aligarh*, pp 269.

Kozlowska, J. (1981). Effect of industrial dusts on the nematode *Panagrolaimus rigidus* in laboratory

nematode *Panagrolaimus rigidus* in laboratory conditions. *Pollut. Ecol. Stud.* **7**: 121-126.

Lagerwerff, J.V. 1967). Heavy metal contamination of soils. In: *Agriculture and Quality of Our Environment. N.C. Brady.* ed. No. **85**: 353-360. Am. Assoc. Adv. Sci. Washington DC.

Lagerwerff, J.V. (1971). Uptake of cadmium, lead, zinc, by radish from soil and air. *Soil Sci.* **111**: 129-133.

Lagerwerff, J.V. and Brower, D.L. (1974). Effect of smelter on the agricultural conditions in the surrounding environment. *Proc. Symp. Trace Substances in Environmental Health* 8th Univ. of Missouri, Columbia, 203-212.

Manning, W.J., (1975). Interactions between air pollutants and fungal, bacterial, and viral plant pathogens. *Environ. Pollut.* **9**: 87-90.

Mathess, G. (1972). Bleigehalte in Gestein, Boden and Grundwrasser. *In : Aurand K (ed) Blei und Umwelt. Berlin Dahlem (Ver Wasser, Boden - Lufthygiene)*, pp 21-27.

Mathess, G. (1974). Heavy metals as trace constituents in natural and polluted Groundwaters. *Geol. Mijnbouw* **53**: 149-155.

Miller, J.E.; Hassett, J.J. and Koeppe, D.E. (1976). Uptake of cadmium by soybean as influenced by soil cation exchange capacity pH and available phosphorus. *J. Environ. Qual.* **5**: 157-160.

Mojtahedi, H. and Lownsbery , B.E. (1976). The effects of ammonia generating fertilizer on *Criconemoides xenoplax* on pot cultures. *J. of Nematology* **8**: 306-309.

Mudd, J. B. and Kozlowski, T. E. (1975). Responses of plants of air pollution. *Academic Press, New York.* pp. 383.

Mudry, T.; Haight, M. and Pasternak, J. (1982). The effects of some heavy metals on the kinetics of pharyngeal pumping in *Panagrellus silusiae Nematologica* **28**: 12-20.

Neuschulz, N. und Kampfe, L.(1980). Das Reporduktions potential von *Caenorhabditis briggsae* unter

unterschiedliche nematizide rinftusse. 5. *Vortr . tag. Zu Aktuellen Problemen der Phytonematology, Rostock, GDR* 1980, S. 46-55.

O' Bannon, J.H. and Santo, G.S.(1981). Influence of volcanic ash on infectivity and reproduction of two species of *Meloidogyne*. *J. of Nematology* **13**(3). 409-412.

Oden, S. (1968). The acidification of air and precepitation and its consequences on the natural environment. Nat. Sci. Res. Coun., Bull No.1, Ecology Committee, Swedish National Science Research Council Stockholm.

Ohba K. and Ishibashi, N. (1984). A nematode *Caenorhabditis elegans* as test organism for nematocide evaluation. *Nippon Noyaku Gakkaishi* **9**(1): 91-96.

Ouden DEN, H. (1958) A new method for culturing plants enabling the observation of nematodes on growing roots. *Tydschr. Plziekt.* **64**: 269-272.

Pansey, V.G. and P.V. Sukhatme (1978). Statistical methods for agricultural workers (Revised by P.V. Sukhatma and V.V. Amble). I.C.A.R., New Delhi.

Parvatha Reddy, P. and Singh, D.B. (1981). Assessment of yield loss in okra, brinjal, french bean and cowpea due to root-knot nematode. *Proc. Third Intn. Symp. Pl. Path.*, New Delhi, pp. 93-94.

Paul, M.T. and Leon, W.B. (1984). Zinc-mediated hatching of eggs of soybean cyst nematodes, *Heterodera glycines*. *Journal of Chemical Ecology* **10**: 361-372.

Pell, E.J. (1979). How air pollutants induce disease. *In: Plant Disease-An Advanced Treatise* (Eds. J.G. Horsefall, and E.B. Cowling) Vol. IV pp. 273-292. Academic Press, New York San Francisco, London.

Pitcher, R.S. and Mc Namara, D.G. (1972). The toxicity of low concentrations of silver and cuperic ion to three species of plant parasitic nematodes. *Nematologica* **18**: 385-390.

Samoiloff, M.R. (1980). Action of chemical and physical agents on free-living nematodes. *In: Nematodes as Biological Models* (B.M. Zuckermann, Ed.), Vol.2, pp. 81-98. Academic Press, New York/London.

Samoiloff, M.R.; Bell, J.; Birkhotz, D.A.; Webster, G.R. B.; Arnott, E.G.; Pulak, R. and Madrid, A. (1983). Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. *Environ. Sci. Technol.* **17**: 329-334.

Samoiloff, M.R. and Bogaert, T. (1984). The use of nematodes in marine ecotoxicology. *In Ecotoxicological Testing for the Marine Environment* (G. Peroone, E. Jaspers, and Chr. Claus, Eds.) part 1, pp. 407-426. State University of Ghent Belgium.

Samoiloff, M.R.; Schulz, S.; Jordan, Y.; Denich, K. and Arnott, E. (1980). A rapid simple long-term toxicity assay for aquatic contaminants using the nematode *Panagrellis redivivus*. *Canad. J. Fish Aquat. Sci.* **37**: 1167-1174.

Sasser, J.N. (1979). Economic importance of *Meloidogyne* in tropical countries. In- *"Root-knot nematodes (Meloidogyne* spp.) Systemics, Biology and Control (Eds. - F. Lamberti and C.E. Taylor) Academic Press, London, pp. 359-374.

Sasser, J.N.; Carter, C.C. and Hartman, K. (1984). "Standardization of host suitability studies and reporting of resistance to root-knot nematodes". *Coop. Pub. Dept. Plant Pathol., N.C. State Univ., and USAID, Raleigh.* pp.7.

Sasser, J.N. and Freckman, D.W. (1987). A world perspective on Nematology: The role of the society. In-"*Vistas on Nematology*" (Eds. - J.A. Veech and D.W. Dickson), Soc. Nematologists, Inc. Hyattsville, pp. 7-14.

Schöttler, U. (1972). Hydrochemische Untersuchung von Sickerwässern unterhalb von Abfallablagerungen, ihr Verhalten in Untergrund und Methodon Zur statistischen Darstellung. Thesis, Aachen.

Schraufnagel, F.H. (1962). Ridge and furrow irrigation for industrial waste disposal. *Sewage and Ind. Wastes* **34**: 1117-1132.

Shafer, S.R.; Koenning, S.R. and Barker, K.R. (1992). Interactions of simulated acidic rain with root-knot or cyst nematodes on soybean, *Phytopathology* **82**: 962-970.

Sharma, N. and Trivedi, P.C. (1993). Studies on the biology of *Heterodera cajani* in three cultivars of *Sesamum indicum*. *J. Indian Bot. Soc.* **73**: 1-2.

Shew, B.B.; Reinert, R.A. and Barker, K.R. (1982). Response of tomatoes to ozone, sulphurdioxide and infecton by *Pratylenchus penetrans*. *Phytopathology* **72**:822-826.

Shriner, D.S. (1978). Effects of simulated acidified rain on host-parasite interactions in plant diseases. *Phytopathology* **68**: 213-218.

Simpkin, K.G. and Coles, G.C. (1981). The use of *Caenorhabditis elegans* for anthelmintic screening. *J.Chem. Technol. Biotechnol.* **31**: 66-69.

Singh, S.K. (1989). Studies on interaction of air pollutants and root-knot nematodes on some pulse crop. *Ph.D. Thesis, A.M.U., Aligarh*,pp.382.

Singh, R.P.; Saxena, S.K. and Kumari, K. (1991). Effect of flyash on the growth of tomato plants, nematode population and available nutrients in soil. *Intern. J. Trop. Agric.* **9**(2): 88-92.

Southey, J.F. (1986). *Laboratory methods for work with plant and soil nematodes. Min. Agri. Fish. Food, HMSO, London*, pp. 202.

Southey, J.F. and Samuel, G.G. (1954). Potato root eelworm. I.A. review of the situation; 2 Research in progress. *Min. Agric. Fish.* N.A.A.S.; pp,12.

Stapel, C. (1953). Plantasygd ommenes Ogskadedyrenes konamike betydring i land brudget. *Tidsskr. Landokon Yr.* 229-244.

Sturhan, D. (1986). Influence of heavy metals and other elements on soil nematodes. *Revue Nematol.* **9**: 311.

Sturhan, D (1987) Nematodes as potential indicators of heavy metals Nematodes in natural system: A status report of a workshop held at the department of Nematology. Agric. Univ. Wageningen. The Netherlands Dec. 16-18, pp 41.

Taylor, A.L (1967) "Introduction to research on plant nematology" Food and Agriculture Organization of the United Nations, Rome, pp. 133.

Temple, P.J and Bisessar, S (1981). Uptake and toxicity of nickle and other metals in crops grown on soil contaminated by a nickle refinery. *J. Plant Nutr.* 3 473-482

Tietjen, J.H and Lee, J.J. (1984). The use of free-living nematodes as a bioassay for estuarine sediments *Mar. Environ. Res.* **11**: 233-251.

Tyler, G. (1974). Heavy metal pollution and soil enzymatic activity. *Plant Soil* **41**: 303-311.

Vaituzis, A , Nelson, J.D.; Wan, L.W. and Colwell, R.R. (1975) Effect of mercuric chloride on growth and morphology of selected strains of mercury resistant bacteria. *Appl. Microbiol.* **29**: 275-286.

Van Berkum, J.A. and Seshadri, A.R. (1970). Some important nematode problem in India. *10th Int. Nematol. Sym. of European Society of Nematologist,* Pascare, Holia .

Van Gundy, S.D. and Thomason, I.J. (1962). Factors influencing storage life of *Hemicycliophora arenaria, Pratylenchus scribneri and Trichodorus christiei* (Abstr.). *Phytopathology* **52**: 366-367.

Van Kessel, W.H.M.; Brocades Zaalberg, R.W. and Seinen, W. (1989). Testing environmental pollutants on soil organisms: A simple assay to investigate the toxicity of environmental pollutants on soil organisms, using $CdCl_2$ and nematodes. *Ecotoxicology and Environmental Safety* **18**: 181-190.

Vranken, G. and Help, C. (1986). Toxicity of copper , mercury and lead to a marine nematode. *Mar. Pollut. Bull.* **17**: 453-457.

Vranken, G.; Vanderhaeghen, R. and Help, C. (1985). Toxicity of cadmium to free-living marine and

microphthalma, Monhystera disjuncta, Pellioditis marina). Diseases of Aquatic Organism **1**: 49-58.

Weber, D.E.; Reinert, R.A.; and Barker, K.R. (1979). Ozone and Sulfur dioxide effects on reproduction and host- parasite relationships of selected plant parasitic nematodes. *Phytopathology* **69**: 624-628.

Weiss, B. and Larink, O. (1991). Influence of sewage sludge and heavy metals on nematodes in an arable soil. *Biology and Fertility of soils* **12**(1): 5-9.

Wood, F.A. (1968). Source of plant pathogenic air pollutant. *Phytopathology* **58**: 1075-1084.

Zullini, A. and Peretti, E. (1986). Lead pollution and moss -inhabiting nematodes of an industrial area. *Water, Air and Soil Pollution* **27**: 403-410.